Physiological Zoölogy

APRIL, 1945

[illegible] Humidity on Animal Life . . . . . *Daniel Ludwig* [illegible]

[illegible] of *Tenebrosus*. III. Sex Differences
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[illegible] Zoölogy . . . . . [illegible]

[illegible] Frederic Daniels, The Permeability of Natural [illegible]

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

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Physiological Zoölogy is published quarterly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. The subscription price is $10.00 a year; the price of single copies is $3.00. Orders for service of less than a full year will be charged at the single-copy rate. Postage is prepaid by the publishers on all orders from the United States and its possessions, Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Guatemala, Haiti, Republic of Honduras, Mexico, Morocco (Spanish Zone), Nicaragua, Panama, Paraguay, Peru, Rio de Oro, El Salvador, Spain (including Balearic Islands, Canary Islands, and the Spanish Offices in Northern Africa; Andorra), Spanish Guinea, Uruguay, and Venezuela. Postage is charged extra as follows: for Canada and Newfoundland, 20 cents on annual subscriptions (total $10.20), on single copies 5 cents (total $3.05); for all other countries in the Postal Union, 48 cents on annual subscriptions (total $10.48), on single copies 12 cents (total $3.12). Patrons are requested to make all remittances payable to The University of Chicago Press in United States currency or its equivalent by postal or express money orders or bank drafts.

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Business correspondence should be addressed to The University of Chicago Press, Chicago 37, Ill.

Communications for the editors and manuscripts should be addressed to W. C. Allee, Managing Editor of PHYSIOLOGICAL ZOÖLOGY, The University of Chicago, Chicago 37, Ill.

The articles in this journal are indexed in the *International Index to Periodicals*, New York, N.Y., the *Quarterly Cumulative Index Medicus*, and *Biological Abstracts*.

Applications for permission to quote from this journal should be addressed to The University of Chicago Press, and will be freely granted.

Entered as second-class matter October 27, 1928, at the post-office at Chicago, Illinois, under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in United States Postal Act of October 3, 1917, Section 1103, amended February 28, 1925.

PHYSIOLOGICAL ZOÖLOGY

Volume XVIII APRIL 1945 Number 2

THE EFFECTS OF ATMOSPHERIC HUMIDITY ON ANIMAL LIFE

DANIEL LUDWIG

Department of Biology, University College, New York University

THE present discussion is limited to the experimental work on terrestrial invertebrates and those lower vertebrates which are normally exposed to the effects of atmospheric humidity. The bulk of the literature on this subject deals with the terrestrial arthropods among the invertebrates and with adult amphibians among the vertebrates. A portion of the work on the invertebrates was covered in the excellent reviews by Uvarov (1931) and Buxton (1932*a*); on amphibians, by Adolph (1933). Many of the results obtained with terrestrial arthropods are complicated by the fact that these organisms may drink water and also obtain moisture from their food. In many cases this difficulty has been eliminated by the use of nonfeeding stages, such as the egg and pupa, and by the use of unfed individuals of the feeding stages. No attempt has been made to include a discussion of water balance in the higher vertebrates. These forms are, to a great extent, independent of atmospheric humidity, since the water content of their bodies is controlled by the water ingested and by the water eliminated through the excretory organs. The experiments on this phase of the problem have been adequately discussed by Adolph (1943) in his monograph *Physiological Regulations*.

Atmospheric humidity affects the organism by modifying its water content. Some forms are hygroscopic and absorb water from moist air. This property has been demonstrated for the potato beetle, *Leptinotarsa decemlineata* (Breitenbrecher, 1918); for the hibernating nymphs of the grasshopper *Chortophaga viridifasciata* (Bodine, 1921); for the growing nymphs and adults of the latter species (Ludwig, 1937); and for the meal worm *Tenebrio molitor* (Buxton, 1930). Buxton found that the meal worm gains weight when starved at a relative humidity of 90 per cent; and in Ludwig's experiments grasshopper nymphs and adults gained weight when starved at relative humidities above 82 per cent. Other forms lose water at all humidities, including saturated air. The loss of water from the frog in saturated air was demonstrated by Durig (1901) and by Adolph (1932). Similar observations were made on insects by Evans (1935), Theodor (1936), Ludwig and Landsman (1937), and Ludwig (1942 and 1943).

Most organisms lose water when exposed to low relative humidities; and, in general, the rate of water loss is greater,

the lower the humidity. However, rate of evaporation is determined by relative humidity only when the temperature is constant. For this reason, many investigators have proposed the use of the vapor-pressure deficit or the saturation deficiency, rather than the relative humidity, as a measure of the moisture content of the atmosphere (Buxton, 1930, 1931*a;* Mellanby, 1932*b;* Anderson, 1936). Anderson pointed out that, since the moisture-holding capacity of the air is greater at higher temperatures, the same relative humidity at different temperatures indicates very different moisture conditions. He further stated that areas having the same vapor-pressure deficits influence evaporation rates in the same way whether temperatures are identical or not. Mellanby (1935) proposed the "law of saturation deficiency," which he stated as follows:

> If individual insects of the same species, in identical morphological and physiological states, are exposed to atmospheres with different humidities, then the rates at which the insects lose water will be proportional to the saturation deficiency, provided that the saturation deficiency is not above a maximum figure which is peculiar to the species.

Mellanby added two corollaries:

> (1) If the rate of loss of water from different individual insects does not appear to be proportional to the saturation deficiency of the air, then their morphological and physiological states cannot be the same. (2) When insects are exposed to atmospheres of different humidity, if there appears to be no direct relation between the length of time which they survive and the saturation deficiency, then other lethal factors, besides desiccation, must be at work.

However, a literature survey has shown that the rates of water loss from insects at different humidities are not proportional to the saturation deficiency over a range of temperatures. At comparable saturation deficiencies the rate of water loss is usually less at low than at high temperatures. This relationship has been demonstrated by Mellanby (1935), who used figures from Bacot and Martin's (1924) work on the rat flea *Xenopsylla cheopis;* by Evans (1934) for the eggs of the blowfly *Lucilia sericata;* by Johnson (1940*a*), who used Mellanby's (1932*b*) figures for the bedbug *Cimex lectularius;* and by Ludwig and Anderson (1942) for the eggs of four species of saturniid moths. Ludwig and Anderson suggested that elevation of the temperature might bring about this effect by accelerating the rate of development. The faster developmental rate at the higher temperature would result in a more rapid production of metabolic water and hence a more rapid loss of water by evaporation. According to Ramsay (1935*a*), the rate of diffusion increases as the temperature is increased; and as a result, at a given saturation deficiency, evaporation would be greater at the higher temperature. He also suggested (1935*b*) that high temperature may increase the permeability of the cuticle to water, thus increasing the rate of evaporation. A departure from the saturation deficiency law is found in those forms which are able to conserve metabolic water. Furthermore, those animals which are hygroscopic do not lose water in accordance with the saturation deficiency law at high relative humidities.

Leighly (1937) and Thornthwaite (1940) have shown by the application of physical principles that the rate of evaporation is not always proportional to the saturation deficiency. When the temperature of the evaporating surface exceeds that of the overlying air, it is possible for water to evaporate into saturated air where the saturation deficiency is zero. This explanation was used by Adolph (1932) to account for the loss of water from the frog in saturated air. The metabolism of the frog raises its temperature

above that of the air, thus establishing a vapor-pressure gradient and permitting evaporation to occur. Hence, the rate of evaporation is proportional to the saturation deficiency only when no temperature gradient exists.

Because of the above limitations to the use of "saturation deficiency" and of the more extensive use of "relative humidity" as a measure of the moisture content of the air, the latter term will be employed in this discussion. Saturation deficiencies may be obtained by reference to a conversion chart of the type published by Buxton (1930).

OPTIMAL HUMIDITIES

For each species there is a range of humidities which may be considered optimal for certain physiological processes. Within this optimal range, development may occur more rapidly, or more individuals may be able to complete development than at other humidities. In many cases, at the optimal point the organisms are able to survive a much greater range of temperature than at other humidities. In still other cases, the reproductive capacity is greatest at the optimal humidity.

In Table 1 are listed the optimal humidities determined for various terrestrial arthropods at particular temperatures. With the exception of the isopods, the most favorable conditions for most species are found in the upper part of the humidity range (70–90 per cent relative humidity). There appears to be a correlation between the normal environment and the humidities which are optimal for survival. Those forms which live on stored grains at rather low humidities are able to survive in relatively dry air. Thus the egg of the flour beetle *Tribolium confusum* survives better at humidities ranging from 1 to 75 per cent than in moist air. The pupae of the same species survive best at humidities ranging from 0 to 25 per cent. The eggs of the bean beetle *Bruchus obtectus* and eggs and larvae of the southern cowpea weevil, *Callobruchus maculatus*, are intermediate in their moisture requirements. The preimaginal stages of the latter species survived better at 44 per cent than at the other humidities employed, when the temperature was 30° C.

For most species, saturated air is above the optimal humidity for survival because it favors the growth of parasitic fungi. This is particularly true of the egg and pupal stages of insects. Hefley (1928) stated that the mortality of pupae of the moth *Protoparce quinquemaculata* at high humidities was due to fungi and bacteria; while the parasite *Winthemia quadripustulata* developed best in moist air because it was resistant to bacterial and fungal diseases. Parker (1930) found that excessive moisture, because it provides ideal conditions for the development of fungal diseases of grasshoppers, is indirectly one of the most important, if not *the* most important, natural checks upon grasshopper abundance. The nymphs die in large numbers during periods of wet, cloudy weather, when the relative humidity is close to the saturation-point for days at a time. Sweetman (1938) observed that dampness was injurious to the nymphs of the firebrat *Thermobia domestica*. It caused many of them to drown, others to adhere to their food; and it also permitted fungal growth.

There are several possible explanations for the increased mortality of organisms in atmospheres of low water content. The eggs of scarabaeid beetles, which require contact moisture for their development, lose water very rapidly at low humidities and in the course of a few hours collapse from desiccation. However, the eggs of many insects are cov-

TABLE 1

OPTIMAL HUMIDITIES FOR DIFFERENT SPECIES OF TERRESTRIAL ARTHROPODS

Species	Stage	Optimal Humidity (Per Cent)	Temperature (°C.)	Physiological Process	Author
Order Isopoda					
Porcellio scaber	Adult	100	20–35	Survival	Miller (1938)
	Adult	100	14–18	Survival	Waloff (1941)
Oniscus asellus	Adult	100	14–18	Survival	Waloff (1941)
Armadillidium vulgare	Adult	100	14–18	Survival	Waloff (1941)
	Adult	100	20–35	Survival	Miller (1938)
Meloponorthus pruinosus	Adult	100	20–35	Survival	Miller (1938)
Porcellio laevis	Adult	100	20–35	Survival	Miller (1938)
P. spinicornis occidentalis	Adult	100	20–35	Survival	Miller (1938)
Ligidium gracilis	Adult	100	20–35	Survival	Miller (1938)
Ligia occidentalis	Adult	100	20–35	Survival	Miller (1938)
L. pallasii	Adult	100	20–35	Survival	Miller (1938)
Order Thysanura					
Thermobia domestica	Egg	76–85	37	Survival	Sweetman (1938)
	Nymph	84	37	Developmental rate	Sweetman (1938)
	Adult	84	37	Oviposition	Sweetman (1938)
Order Collembola					
Sminthurus viridis	Adult	100	25	Survival	Davies (1928)
	Nymph	100	30	Survival	Maclagan (1932)
	Adult	80–90	7	Oviposition	Maclagan (1932)
Isotoma viridis	Adult	100	25	Survival	Davies (1928)
Tomocerus vulgaris	Adult	100	25	Survival	Davies (1928)
Entomobrya multifasciata	Adult	100	25	Survival	Davies (1928)
Dicyrtomia minuta	Adult	100	25	Survival	Davies (1928)
Order Orthoptera					
Melanoplus mexicanus	Egg	90	27, 32, 37	Survival	Parker (1930)
	Egg	80	22	Survival	Parker (1930)
Schistocerca gregaria	Adult	50–60	37.8	Oviposition	Hamilton (1936)
	Nymph	55–70	37.8	Developmental rate	Hamilton (1936)
	Egg	100	30	Survival	Bodenheimer (1929)
Locusta migratoria migratorioides	Adult	60–75	37.8	Oviposition	Hamilton (1936)
	Nymph	60–70	32.2	Developmental rate	Hamilton (1936)
	Nymph	60–65	37.8	Developmental rate	Hamilton (1936)
Camnula pellucida	Egg	60–90	27	Developmental rate	Parker (1930)
Order Thysanoptera					
Thrips tabaci	Larva	75–85	10–35	Survival	MacGill (1931)
Order Heteroptera					
Rhodnius prolixus	Egg	70–90	17–33	Survival	Clark (1935)
Cimex lectularius	Egg	75–90	13–35	Survival	Johnson (1940b)
	Egg	70	18	Survival	Geisthardt (1937)
	Nymph	44–77	18	Developmental rate	Geisthardt (1937)
C. rotunda	Egg	93	18	Survival	Geisthardt (1937)
C. rotunda	Nymph	77–93	18	Developmental rate	Geisthardt (1937)
Eurygaster mauro	Nymph	80–100	24–30	Survival	Tischler (1939)
Palomena prasina	Nymph	80–100	24–30	Survival	Tischler (1939)
Dysdercus cingulatus	Egg	82–100	35	Survival	Mehta (1930)
Order Anoplura					
Haematopinus asini	Egg	100	37.8	Survival	Bacot and Linzell (1919)
Order Coleoptera					
Bruchus obtectus	Adult	100	26.6	Oviposition	Headlee (1917)
	Adult	90	25.2	Oviposition	Menusan (1935)
	Egg	50–75	25.2	Survival	Menusan (1934)
	Egg	90	25.2	Developmental rate	Menusan (1934)
Callosobruchus chinensis	Larva	76	30.4	Developmental rate	Utida (1941)
	Larva	76	30.4	Survival	Utida (1941)
C. maculatus	Egg	44–63	30	Survival	Schoof (1941)
	Egg	63	30	Developmental rate	Schoof (1941)
	Preimaginal stages	91	30	Developmental rate	Schoof (1941)
	Preimaginal stages	44	30	Survival	Schoof (1941)
Lasioderma serricorne	Egg	75	32	Developmental rate	Powell (1931)
	Egg	75	32	Survival	Powell (1931)
Tribolium confusum	Egg	1–75	27	Survival	Holdaway (1932)
	Pupa	0–25	27	Survival	Holdaway (1932)
Epilachna corrupta	Egg	76–81	27	Survival	Pyenson and Sweetman (1931)
	Egg	81–100	27	Developmental rate	Pyenson and Sweetman (1931)
Hylotrupes bajulus	Egg	90–95	31.5	Developmental rate	Steiner (1937)
	Egg	90–100	31.5	Survival	Steiner (1937)
	Egg	70–80	19.3	Developmental rate	Steiner (1937)
	Egg	50–60	16.6	Developmental rate	Steiner (1937)

TABLE 1—*Continued*

Species	Stage	Optimal Humidity (Per Cent)	Temperature (°C.)	Physiological Process	Author
Order Cleoptera—*Continued*					
Agriotes lineatus	Egg	100	19	Survival	Subklew (1934)
	Larva	98–100	20	Survival	Subklew (1934)
Calandra granaria	Adult	60–75	20–27.5	Oviposition	Eastham and McCully (1943)
Hypera postica	Egg	55–95	20–30	Survival	Sweetman and Wedemeyer (1933)
	Adult	50–95	27	Oviposition	
	Egg	72–82	8–20	Survival	Andersen (1930)
	Egg	100	10–32	Developmental rate	Andersen (1930)
Ptinus tectus	Preimaginal stages	70	24.7	Developmental rate	Ewer and Ewer (1942)
	Egg	70	25	Developmental rate	Ewer and Ewer (1942)
	Larva	70	24.7	Developmental rate	Ewer and Ewer (1942)
	Pupa	90	27	Developmental rate	Ewer and Ewer (1942)
Anomala orientalis	Egg	100	25.5	Survival	Zwaluwenburg (1937)
Order Lepidoptera					
Protoparce quinquemaculatus	Pupa	0–73.4	27	Developmental rate	Hefley (1928)
Prodenia littoralis	Egg	90–95	29	Survival	Janisch (1930)
Panolis flammea	Egg	65–85	12–22	Survival	Zwölfer (1931)
Dendrolinus pini	Egg	80–85	24	Survival	Kojima (1933)
Malacosoma neustria	Egg	90	11–21	Survival	Bekir (1935)
Callosamia promethea	Egg	76	20–30	Survival	Ludwig and Anderson (1942)
Platysamia cecropia	Egg	56–76	25	Survival	Ludwig and Anderson (1942)
Telea polyphemus	Egg	53–76	30	Survival	Ludwig and Anderson (1942)
Samia walkeri	Egg	60–100	15–20	Survival	Ludwig and Anderson (1942)
	Egg	56–76	25	Survival	Ludwig and Anderson (1942)
Tineola bisselliella	Larva	75	23.9	Developmental rate	Griswold (1944)
	Larva	75	23.9	Developmental rate	Griswold and Crowell (1936)
Agrotis segetum	Pupa	65–85	17–21	Developmental rate	Kozhantchikov (1936)
Order Diptera					
Drosophila ampelophila	Pupa	100	18–19	Developmental rate	Elwyn (1917)
Lucilia sericata	Egg	100	22	Developmental rate	Evans (1934)
	Egg	100	10–37	Developmental rate	Wardle (1930)
	Egg	90–100	37	Survival	Davies and Hobson (1935)
Order Hymenoptera					
Winthemia quadripustulata	Pupa	73.4	27	Developmental rate	Hefley (1928)
Habrobracon juglandis	Egg	80	16–35	Survival	Maercks (1933)
Order Acarina					
Ixodes ricinus	Adult	100	25	Oviposition	MacLeod (1935)
	Nymph	100	30	Developmental rate	MacLeod (1935)
I. ricinus	Nymph	90–95	27.5	Developmental rate	MacLeod (1934)
	Nymph	90	25	Developmental rate	MacLeod (1934)
Halotydeus destructor	Adult	100	18–26	Survival	Solomon (1937)

ered with a chorion which is relatively impermeable to water. The embryos may complete their development normally, but in many cases the larvae are unable to hatch. Ludwig and Anderson (1942) observed that, when the eggs of saturniid moths were exposed to low humidities, many more larvae developed than emerged. In dry air many larvae pierced the shells but died without emerging. Hence, in these eggs the chorion is very efficient in preventing evaporation, retaining this function until pierced by the emerging larvae. Thereupon the larvae lose water so rapidly in dry air that very few of them are able to hatch. Comparable observations were made by Janisch (1930) for the eggs of the moth *Prodenia littoralis*. In some cases the limits of humidity which permit hatching are narrower than those which permit development, indicating that at the lower humidities death occurs because the larvae are so weakened by loss of water that they cannot emerge or because desiccation hardens the chorion to such an extent that the larvae are unable to escape (Pyenson and Sweetman, 1931).

At low humidities survival, during the feeding stages of an organism, is influenced by starvation as well as by desiccation. Ludwig (1937) kept unfed nymphs and adults of the grasshopper *Chortophaga viridifasciata* at different humid-

ities at 25° C. Regardless of humidity, death by starvation occurred in from 5.2 to 6.6 days, but the water content at death varied with the humidity. On the other hand, the time of survival of the larvae, prepupae, and pupae of the Japanese beetle, *Popillia japonica*, varied with the humidity. For the larva it ranged from 3.8 days in dry air to 30.4 days in saturated air. At humidities of 82 per cent and below, death was caused by desiccation, occurring when the water content had been reduced to 55–59 per cent (Ludwig and Landsman, 1937). In general, when factors other than loss of water are excluded, the time of survival at low humidities is determined by the saturation deficiency of the air. A few exceptions to this general statement are discussed by Buxton (1932*a*) and Wigglesworth (1939).

RELATIVE HUMIDITY AND REPRODUCTION

The data included in Table 1 show that humidity influences oviposition in a manner comparable to its effects on other physiological processes. There is usually an optimal humidity at which oviposition is completed in the shortest time. Furthermore, at a certain humidity a larger number of eggs may be produced than at any other humidity value. Hence, humidity modifies the rate of oviposition and the total egg production of the organism. In some species optimal conditions are found in saturated air (Headlee, 1917; Breitenbrecher, 1918; Adolph, 1920; Huber, Neiswander, and Salter, 1928; MacLeod, 1935), but in most species saturated air appears to be somewhat detrimental (Roubaud, 1909; Zwölfer, 1931; Davidson, 1932; Menusan, 1935; Buxton and Lewis, 1935; Hamilton, 1936; Kozhantchikov, 1936; Utida, 1941; Eastham and McCully, 1943). Usually, humidity has a greater effect on the average number of eggs produced than on the duration of the oviposition period.

As indicated in Table 1, closely related forms may differ in the amount of moisture required for oviposition. Hamilton (1936) compared moisture requirements of the grasshopper, *Locusta migratoria migratorioides* and *Schistocerca gregaria*. A relative humidity below 25 per cent stopped oviposition in the former species; below 30 per cent, in the latter species. In these insects the average number of egg pods per female also varied with humidity, the maximum being produced (when the temperature was 100° F.) at 60–75 per cent humidity by *Locusta* and at 50–60 per cent by *Schistocerca*.

Dick (1937) found considerable differences in the moisture requirements for egg production in three species of Coleoptera: *Tribolium confusum*, *Tenebrio molitor*, and *Dermestes vulpinus*. *Tribolium*, when kept in dry air and fed flour previously heated at 100° C. to a constant weight, was able to lay a daily average of 3.13 eggs per female. When fed flour equilibrated to 65 per cent humidity, this average was increased to 5.6 eggs. *Dermestes* must drink water if it is to lay normally. When adult females were kept in fish meal equilibrated with an atmosphere of 73 per cent humidity, but not allowed to drink water, they laid an average of 30.4 eggs; while others, kept under the same conditions but allowed to drink, laid an average of 567 eggs. *Tenebrio* does not need to drink water for normal oviposition. However, the number of eggs deposited varied with humidity. At 20 per cent an average of only 4 eggs was laid; at 65 per cent, this average was 102.4. Ewer and Ewer (1942) observed that the beetle *Ptinus tectus*, a pest of stored products, also must drink water in order to lay the normal number of eggs.

Headlee (1917) observed that no reproduction occurred when the bean beetle *Bruchus obtectus* was exposed to humidities of 26 per cent or below. In this insect, lowering the humidity reduced the total number of eggs laid; but the amount of this reduction was not sufficient to explain the smaller number of progeny at low humidities. Headlee believed that under these conditions the larvae were destroyed by dehydration before they were able to penetrate the bean and begin feeding.

Breitenbrecher (1918) made some interesting observations on the behavior and oviposition of the potato beetle, *Leptinotarsa decemlineata*, under different moisture conditions. When beetles were buried in moist soil, they became positive to light and negative to gravity. When these beetles were subsequently placed outdoors on the top of soil in which food plants were growing, they began to feed and to lay eggs. Under similar conditions, however, beetles which had previously been buried in dry soil were positive to gravity; they burrowed into the ground, remained there until the summer rains, then emerged and laid eggs. Breitenbrecher found that a low rate of evaporation encouraged oviposition and that a high rate retarded it.

Eastham and McCully (1943) pointed out that in the granary weevil *Calandra granaria* the rate of oviposition varies with the saturation deficiency rather than with the relative humidity. For all temperatures the optimal saturation deficiency is 7.4 mm. Hg. It is obtained at 60 per cent relative humidity when the temperature is 20° C. and at 75 per cent at 27.5° C. At any given temperature the total egg production per female varies inversely with the saturation deficiency. However, in their experiments they did not use relative humidities above 80 per cent.

Ludwig (1942, 1943) subjected the pupal stages of the Luna moth, *Tropaea luna*, and the Cynthia moth, *Samia walkeri*, to different humidities and counted the number of eggs laid by adults which emerged from the treated pupae. He selected these insects because the adults take in no food or water, which might influence egg production. Furthermore, they begin oviposition within a day or two of emergence and complete the process within a few days. Subjecting Luna pupae to humidities ranging from 56 to 100 per cent at 25° C. had no effect on the average number of eggs laid by each emerged female. However, when pupae were subjected to lower humidities, there was a marked reduction in number of eggs, only 51.2 eggs per female being produced when pupae were kept in dry air, as compared with an average of 183.9 when pupae were kept in saturated air. Cynthia pupae are more resistant to desiccation than Luna pupae and can be subjected to much lower humidities and desiccated to a much greater extent before there is a reduction in egg production. The number of eggs laid by mated Cynthia moths which had completed pupal development at different humidities averaged 58 at 0 per cent, 192.3 at 16 per cent, and more than 200 at higher humidities. However, a study of the ovaries of the exhausted females showed that the total number of eggs formed was not greatly reduced by subjecting pupae to low humidities. Excessive dehydration evidently interferes with the mechanism of oviposition.

The degree of humidity definitely influences the reproductive capacity of insects. Usually the higher humidities are more favorable, but in most cases the optimal value is below saturation. A few insects must drink water in order to lay their normal number of eggs. In the saturniid moths, subjecting the pupae

to low humidities reduces the number of eggs produced by the moths emerging from treated pupae. Low humidity may reduce the number of eggs laid by interfering with the mechanism of oviposition.

THE INTERACTION OF TEMPERATURE AND HUMIDITY

Shelford (1914) summarized the interrelationship existing between temperature and humidity as follows:

> (*a*) A moist cold atmosphere (very low evaporation) causes body temperature to fall more rapidly than a dry cold one at the same temperature, because of the more rapid conduction of heat. Such a fall in temperature decreases metabolism of cold blooded animals, and increases metabolism of warm blooded animals within their capacity for heat regulation. In a dry cold atmosphere the heat loss is less pronounced because of the less rapid conduction of heat. (*b*) In a dry warm atmosphere (high evaporation) rapid evaporation keeps down peripheral temperature, and prevents death from overheating and destructive metabolism in cold blooded animals, and makes possible body temperature regulation and thus prevents heat stroke and death in warm blooded animals. In a moist warm atmosphere, death and heat stroke occur because of lack of evaporation and lack of peripheral cooling in the case of warm blooded animals even when the surrounding temperature is at or below the normal body temperature.

In a few insects the rate of development is independent of relative humidity at all temperatures studied, as demonstrated for eggs of the following insects: two species of bedbugs, *Cimex lectularius* and *C. rotunda* (Geisthardt, 1937), the bedbug *C. lectularius* (Johnson, 1940*b*), the firebrat *Thermobia domestica* (Sweetman, 1938), and the clothes moth *Tineola bisselliella* (Griswold, 1944). Other studies have shown the rate of development to be independent of relative humidity only in a particular temperature range, while above and below this range the developmental rate is retarded at low humidities. According to Maercks (1933), the developmental rate-humidity curve for the egg of the wasp *Habrobracon juglandis* is a horizontal straight line at 30° C.; while at 13.5° C. the developmental time is lengthened at both low and high humidities, as compared with the optimal point. Ludwig and Anderson (1942) showed that the development of the eggs of the Promethea moth, *Callosamia promethea*, and the Polyphemus moth, *Telea polyphemus*, required the same time at low as at high humidities when the temperature was 25° C., but for the egg of the Cecropia moth, *Platysamia cecropia*, this temperature was 20° C. Low humidity retarded development at temperatures above and below these optima. Finally, in some species the rate of development is retarded by low humidities regardless of temperature. However, in these species the humidity effect is less at certain temperatures, usually considered optimal for development, than it is above and below these temperatures. This observation was made by Ludwig and Anderson (1942) with the eggs of the Cynthia moth, *Samia walkeri*. Griswold (1944) observed that humidity had a marked effect on the length of the developmental period (egg to adult) of the clothes moth *Tineola bisselliella*, the time being prolonged by low humidities. This effect was more pronounced when the larval diet was not entirely satisfactory.

Temperature influences the survival of organisms at different humidities. For each favorable temperature there is a definite range of humidities in which it is possible for development to occur. Usually this humidity range is widest at those temperatures which permit the highest percentage survival. The eggs of the bean beetle and of granary insects, which are normally exposed to low humidities, are apparent exceptions. Thus, Menusan (1934) stated that humidity is

not important in determining the viability of the egg of the bean beetle *Bruchus obtectus;* the same percentage hatch at all humidities regardless of temperature; and Ahmad (1936) found that the viability of the egg of the Mediterranean flour moth, *Ephestia kuehniella*, was not affected by humidities from 5 to 92 per cent at temperatures which ranged from 18° to 30° C. For most arthropods, as the temperature is increased above the optimal range for survival, the humidity range which permits development becomes narrower and is limited to the higher humidities (Mehta, 1930; Parker, 1930; MacGill, 1931; Mellanby, 1933; MacLeod, 1934, 1935; Hamilton, 1936; Steiner, 1937; Sweetman, 1938; Tischler, 1939; Johnson 1940*b;* Ludwig and Anderson, 1942). As the temperature is reduced below the optimal range for survival, the humidity range is also restricted, usually being limited to those humidities near the optimum for survival (Pyenson and Sweetman, 1931; Maercks, 1933; Clark, 1935; Ludwig and Anderson, 1942).

The data in Table 1 indicate that the optimal humidity for development and for survival may also shift with the temperature. Parker (1930) found that, when the eggs of the grasshopper *Camnula pellucida* were subjected to 22° C., viability was highest at 80 per cent relative humidity; whereas at temperatures ranging from 27° to 37° C., maximal viability was obtained at 90 per cent. Steiner (1937) noted a shift in the optimal humidity range for the survival of the eggs of the beetle *Hylotrupes bajulis* from 90–100 per cent at 31.5° C. to 50–60 per cent at 16.6° C. Johnson (1930*b*) studied the effects of temperature and humidity on the development and hatching of the eggs of the bedbug *Cimex lectularius*. At 13° C. the optimal humidity range for hatching was from 75 to 90 per cent; at optimal temperatures (16°–30° C.), varying humidity had only a slight effect, the very low humidities being less favorable to both embryonic development and hatching. Above 30° C., mortality occurred early when the humidity was low, and at 37° C., no eggs hatched at any humidity. Ludwig and Anderson (1942) observed that the egg of the Cynthia moth, *Samia walkeri*, survived best at humidities which ranged from 60 to 100 per cent at 15° and 20° C. and from 56 to 76 per cent at 25° C. At the lower temperatures saturated air proved more favorable because these temperatures inhibited the growth of molds. MacLeod (1934) studied the development of the tick *Ixodes ricinus* at different temperature and humidity combinations and made the interesting observation that with decreasing humidities the point at which the greatest developmental velocity is shown tends to move down the temperature scale and that with decreasing temperatures the point of greatest developmental velocity moves down the humidity scale.

The effect of humidity on body temperature.—As was already mentioned, the relative humidity of the atmosphere directly modifies the body temperature of the organism by determining the amount of heat which may be lost by conduction and by the evaporation of moisture and indirectly by changing the rate of metabolism (Shelford, 1914). Koidsumi (1934) believed that the loss of heat from insects is effected almost entirely by evaporation. In the beetle *Anomala* and in the psychid larva *Clania*, between the temperatures of 10° and 30° C., evaporation accounts for 80–100 per cent of the total loss of heat; conduction and radiation, for only 0–20 per cent. Since humidity is very important in controlling water loss, it must be important in regulating the body temperature of insects. Bachmet-

jew (1901) concluded that the body temperature is lower than the environmental temperature at "normal" humidity but is higher than that of the environment in saturated air. In his experiments the body temperature of the hawk moth *Deilephila euphorbiae*, in unmoistened air, lagged behind the external temperature when the latter was gradually increased; e.g., at 47.6° C. the body temperature was only 45.1° C. The difference between the two temperatures became less as the upper fatal limit was approached. Necheles (1924) found that in the cockroach *Blatta orientalis*, at high temperatures, evaporation was increased and the body temperature fell below that of the environment. Between the temperatures of 13° and 23° C. the body temperature ran parallel to that of the air; while above 23° in dry air it was lower than that of the environment. Buxton (1924) showed that in the hot, dry air of deserts the body temperature of certain insects, such as large grasshoppers, exposed to the full radiation of the sun, was lower in living than in dead ones, presumably because of the greater amount of evaporation from the living insects. The tenebrionid beetle *Adesmia ulcerosa*, alive on top of soil with a surface temperature of 38° C., had a body temperature of 36° C.; with soil at 44° C., body temperature was 39.5° C. Dead insects under similar conditions were always at least 2° warmer than living ones. Bodenheimer (1929) reported that the desert locust *Schistocerca gregaria* was able to lower its temperature in dry air at 40° C. Mellanby (1932*c*), repeating the work of Necheles on the cockroach, demonstrated that, when this insect is exposed to moist air at 45° C., its temperature very soon reaches the same value; but if it is exposed to dry air at the same temperature, it can maintain its body temperature at 39° C. for a considerable period. According to Mellanby, meal worms weighing over 100 mg. are able to withstand a higher temperature in dry than in moist air during an exposure of 1 hour. This property is not possessed by small meal worms. Buxton (1933) concluded that the larger the insect, the less surface it has in proportion to its weight, and that at higher temperatures the larger insect will receive proportionately less heat by conduction. To balance this heat, the larger insect is better able to withstand the loss of water by evaporation than is the smaller insect, since the latter would become desiccated to a much greater extent than the former in losing a certain amount of water.

Hall and Root (1930) studied the effects of humidity on the body temperatures of amphibians and reptiles. In atmospheres of low humidities the body temperature of amphibians is much lower than that of the environment because of the rapid loss of water from the surface of the body. On the other hand, the body temperature of reptiles is only slightly changed by similar conditions. The difference between the two classes is associated with the type of integument. Amphibians with a moist skin readily lose water by evaporation. On the other hand, the scaly integument of the reptile greatly increases its power of water retention. Reptiles give up water slowly and resist desiccation for long periods of time. Mellanby (1941) made a number of observations on the body temperature of the frog *Rana temporaria* in relation to the rate of evaporation. When placed in water at temperatures between 0° and 35° C., the frog maintained its body temperature within 0.1° C. of the environment. In unsaturated air the frog was considerably cooler than its environment, as a result of the evaporation of water. In still air (under laboratory conditions; temperature, 21° C.) the frog's

temperature fell about 3° C. in 1 hour. In air moving about 1.9 meters per second the body temperature of the frog was only 0.1° C. above the temperature of the wet-bulb thermometer. Mellanby concluded that under most conditions the amount of metabolic heat produced by a frog is so small that the animal behaves like a nonliving system and its temperature is controlled by external physical conditions.

Relation of humidity to thermal death-point.—In Table 2 are listed the thermal death-points of insects at different humidities. Here the thermal death-point is the minimum temperature which was fatal to all of the insects during the time of exposure.

Beattie (1928) found that the thermal death-point of the blowfly *Calliphora erythrocephala* was definitely influenced by humidity. Saturated and dry air lowered it, while a relative humidity of 70 per cent was optimal. Death in saturated air was due to the inability of the flies to regulate their temperature by evaporation. Savely (1939) determined the thermal death-points of the larvae of several species of beetles which live in dead pine and oak logs. The larva of *Chrysobothris femorata* had the highest thermal death-point (53° C.), and it lost less water than the other forms studied. This species is thermophilic, mating and laying eggs in places exposed to the sun. It is able to survive at high temperatures because of its relatively slow rate of water loss. The larvae of three of the species studied by Savely were able to survive a higher temperature at low than at high humidities. The more rapid loss of water at the low humidities resulted in a reduction of the body temperature. On the other hand, the thermal death-point of *Dendroides bicolor* was not affected by humidity. This insect is so small and has such a large surface area in proportion to its weight, and so much heat is absorbed per unit weight, that the amount of water which can be evaporated from its body is not sufficient to keep its temperature below that of the surroundings.

Buxton (1933) discussed much of the work summarized in Table 2. He divided insects into the following four classes, depending on the effect of humidity on their ability to survive high temperatures.

Class 1: Those insects in which death is due to heat and occurs at the same temperature, irrespective of humidity: the nymphs of *Rhodnius prolixus* and the adults of *Xenopsylla cheopis* and *Cimex lectularius* belong to this class.

Class 2: Those insects which lose so much water in dry air that the temperature which is fatal to them is lower in dry than in moist air: the larva of *Xenopsylla cheopis* and the adults of *Pediculus* and *Lucilia* show this type of reaction in a 24-hour experiment.

Class 3: Those insects which are capable of surviving a higher temperature in dry than in moist air: this reaction is shown by large meal worms. A possible explanation is given above.

Class 4: Those insects which are intermediate: the adult blowfly *Calliphora erythrocephala* is killed by a temperature of 40° C. in 1 hour when the air is saturated, but at 10 per cent relative humidity a temperature of 41° C. is required to produce this effect. (In the writer's opinion, there is no essential difference between insects of Buxton's Classes 3 and 4.)

The effect of relative humidity on survival at low temperatures.—Bachmetjew (1901), who stated that the percentage of "sap" is inversely proportional to the freezing-point, was probably the first to correlate the amount of fluid, or "sap," present in an insect with its freezing-point. The "sap" is the amount of water

TABLE 2

EFFECT OF RELATIVE HUMIDITY ON THE THERMAL DEATH-POINT OF ARTHROPODS

Species	Stage	Relative Humidity (Per Cent)	Thermal Death-Point (°C.)	Exposure (Hours)	Author
Order Orthoptera					
Blatta orientalis	Adult	0	48	?	Necheles (1924)
	Adult	Moist	38	?	Necheles (1924)
	Adult	0	46	1	Gunn and Notley (1936)
	Adult	90	43	1	Gunn and Notley (1936)
Periplaneta americana	Adult	0	45	1	Gunn and Notley (1936)
	Adult	90	42	1	Gunn and Notley (1936)
Blatella germanica	Adult	0	45	1	Gunn and Notley (1936)
	Adult	90	43	1	Gunn and Notley (1936)
Blatta orientalis	Adult	0	34–36	24	Gunn and Notley (1936)
	Adult	90	37–38	24	Gunn and Notley (1936)
Periplaneta americana	Adult	0	37–39	24	Gunn and Notley (1936)
	Adult	90	37–39	24	Gunn and Notley (1936)
Blatella germanica	Adult	0	33–39	24	Gunn and Notley (1936)
	Adult	90	37–39	24	Gunn and Notley (1936)
Order Anoplura					
Pediculus humanus	Adult	0	46.2	1	Mellanby (1932c)
	Adult	90	46.8	1	Mellanby (1932c)
	Adult	0	32.8*	24	Mellanby (1932c)
	Adult	90	38.2*	24	Mellanby (1932c)
Order Siphonaptera					
Xenopsylla cheopis	Adult	0	40.6	1	Mellanby (1932c)
	Adult	90	40.7	1	Mellanby (1932c)
	Adult	0–90	37.8*	24	Mellanby (1932c)
	Larva	0–90	39.5*	1	Mellanby (1932c)
Order Heteroptera					
Cimex lectularius	Adult	0–90	43.5	1	Buxton (1933)
	Adult	0–90	39	24	Buxton (1933)
Rhodnius prolixus	Nymph	0–90	43	1	Buxton (1931*b*)
	Nymph	0–90	40	24	Buxton (1931*b*)
Order Coleoptera					
Chrysobothris femorata	Larva	10–15; 95–100	53	2	Savely (1939)
Romaleum atomarium	Larva	10–15	50	2	Savely (1939)
	Larva	95–100	45	2	Savely (1939)
Monochammus titillator	Larva	10–15	51	2	Savely (1939)
	Larva	95–100	49	2	Savely (1939)
Acanthocinus nodosus	Larva	10–15	48	2	Savely (1939)
	Larva	95–100	46	2	Savely (1939)
Dendroides bicolor	Larva	10–15; 95–100	42	2	Savely (1939)
Tenebrio molitor	Larva under 30 mg.	0–90	41.8*	1	Mellanby (1932c)
	Larva over 100 mg.	0	42.8*	1	Mellanby (1932c)
	Larva over 100 mg.	90	41.8*	1	Mellanby (1932c)
	Larva over 100 mg.	0–90	38.5*	24	Mellanby (1932c)
Epilachna corrupta	Adult	1–100	42.5	3	Miller (1930)
	Larva	1–100	42.5	3	Miller (1930)
Order Diptera					
Lucilia sericata	Larva	0–90	43.2*	1	Mellanby (1932c)
	Larva	0	32*	24	Mellanby (1932c)
	Larva	90	36*	24	Mellanby (1932c)
Piophila casei	Larva	0	46	24	Smart (1935)
	Larva	60	48	24	Smart (1935)
	Larva	100	46	24	Smart (1935)
	Larva	0–100	52.5	1	Smart (1935)
Glossina submorsitans	Adult	0–90	44	1	Buxton and Lewis (1935)
Calliphora erythrocephala	Adult	10	41	1	Beattie (1928)
	Adult	70	43	1	Beattie (1928)
	Adult	80	42	1	Beattie (1928)
	Adult	100	40	1	Beattie (1928)

* Values obtained from graphs.

which may be lost by desiccation and thus represents the difference in weight between a normal insect and a desiccated one. Bodine (1921, 1923) found a seasonal variation in the water content of the grasshopper *Chortophaga viridifasciata*. In the fall the water content gradually drops to a low of approximately 65 per cent, remaining there until spring, when it gradually rises to a value of approximately 75 per cent. Bodine pointed out that the ability of the grasshopper to resist cold during the winter is associated with its low water content. Payne (1926) studied the cold resistance of a number of insects and also showed that the ability to resist low temperatures is associated with low water content. She was able to make insects cold-hardy by experimental dehydration or by exposure to low temperatures. In 1927 she demonstrated that cold-resistant forms can be made nonresistant by exposure to high humidities or to high temperatures. In 1928 she exposed larvae of the Japanese beetle, *Popillia japonica*, to different relative humidities and found that at low humidities the conductivity of the body fluids increased. The higher conductivity indicated a greater salt content, owing to loss of water, and was associated with an increased cold-hardiness. Robinson (1927) showed that in cold-hardy forms the hydrophilic colloids of the body take up water on exposure to low temperature, thus reducing the amount of freezable water in the insect. Sacharov (1930) studied the water content and freezing-points of a few insects, using the eggs, larvae, and adults. He concluded that

> cold hardiness in insects depends on the minimum quantity of water and the accumulation of fat in the organism. All hibernating stages of insects prepare themselves to withstand cold by reducing the quantity of free, easily freezable water in their tissues.

According to Payne (1929), the cold-hardiness of the eggs and larvae of the moth *Hemerocampa leucostigma* is directly related to the absolute humidity of the air, according to the equation

$$T = K\left(\frac{1}{H}\right),$$

where T is the survival temperature in degrees centigrade, H is the absolute humidity in millimeters of vapor pressure, and K is a constant. Decker and Andre (1936) have shown that short exposures to low relative humidities resulted in a loss of water, which increased the cold resistance of the chinch bug, *Blissus leucopterus*.

However, not all insects are able to regulate their moisture content on exposure to low temperatures, and consequently they do not show a seasonal increase in cold resistance. This group includes granary insects, aquatic insects, and the honeybee (Robinson, 1926, 1927; Payne, 1927; and Sacharov, 1930). Furthermore, some insect eggs are as resistant to low temperatures when moist as when dry (Sacharov, 1930; Parker, 1930; and Salt, 1936).

From the foregoing discussion it is evident that a decrease in water content increases the cold resistance of insects. Hence, one would expect to find conditions prevalent in the fall which would tend to favor the loss of water from the insect. Hodson (1937), in his monograph *Some Aspects of the Role of Water in Insect Hibernation* has given a comprehensive discussion of the importance of moisture in determining the cold-hardiness and the survival of insects during winter conditions, as found in St. Paul, Minnesota. After a study of field conditions, Hodson stated that factors which hasten the dissipation of moisture are less important during the fall and winter

than during the spring and summer. In the fall there is a gradual decrease in temperature, saturation deficiency, and the percentage of sunlight. At the same time, there is a slight increase in relative humidity. Such a combination of conditions tends to slow up the rate of evaporation of water from the soil and other places in which insects hibernate. An examination of the hibernacula of arthropods indicates that during hibernation these organisms are often exposed to a saturated atmosphere and may even be in contact with liquid water. Hodson studied the moisture preference of insects entering hibernation and found that most of the species studied preferred moist situations. A moist environment probably serves as a protection during very cold weather, since the latent heat of water tends to prevent a too rapid subzero drop in temperature. The box-elder bug, *Leptocoris trivittatus*, shows a preference for dry leaves and dry soil at the time of hibernation. This preference may be correlated with the relation existing between the conditioning of the insects at different relative humidities and their survival at low temperatures. When exposed to moderately low temperatures, their chances for survival decrease as the conditioning atmospheric moisture decreases. However, when exposed to −14° C., only at humidity values above 60 per cent is there an increase in resistance to low temperature as the conditioning relative humidity is decreased. After conditioning in drier air the animals are killed more easily by very low temperatures. The following moisture conditions were found to exist for the beetle *Ceratomegilla fuscilabris:* "A wet environment was most favorable for survival at temperatures above freezing; a moderately wet environment was most desirable at sub-zero temperatures; a dry environment was unsuitable at all times." Hodson found that soil moisture did not affect the resistance to low temperatures unless it was present in quantities greater than that which could be absorbed by the soil. However, contact moisture may increase mortality when insects are exposed to low temperatures, probably because the body fluids are actually inoculated by the freezing of water on the surface of the body, thus preventing the protective phenomenon of undercooling of the tissues.

Atmospheric humidity modifies the cold resistance of insects by controlling the amount of water which can be lost from the tissues. Any process which reduces the amount of free freezable water in the insect increases its cold-hardiness, provided this reduction is not excessive. Either excessive or insufficient dehydration is harmful. A moist environment is beneficial during exposure to low temperatures because the latent heat of water prevents a very rapid drop in temperature, particularly at values below zero.

Since moisture is an important factor in the cold resistance of insects and since the water content of the insect may be decreased during exposures to low temperatures and during entrance into hibernation, it follows that moisture must also be an important factor determining emergence from hibernation. Breitenbrecher (1918) found that the emergence of the potato beetle, *Leptinotarsa decemlineata*, from hibernation was determined by the environmental complex. When water was added to the medium surrounding hibernating beetles, provided the soil temperature was above 14° C., emergence from hibernation resulted. However, the potato beetle was not able to absorb much water at temperatures below 12° C. Fink (1925) also reported that an adjustment of water content is important in the emergence of the potato

beetle from hibernation. In his experiments contact moisture was necessary. He believed that the water imbibed during recovery aids in the elimination of inert substances from the digestive tract prior to feeding. Townsend (1926) was able to hasten the end of hibernation of codling-moth larvae by soaking them at intervals (2 hours per week), regardless of whether the temperatures was 10° or 30° C. He believed that the dilution of the body fluids speeded enzyme action and hence the general activity of the insect. Hodson (1937) noticed that during the spring the water content of hibernating *Leptocoris trivittatus* increased when the bugs were in contact with wet leaves. Other bugs in saturated air showed no such increase until activity and feeding had occurred. He exposed hibernating bugs to contact moisture at 2° and 6° C. for 8 days. The gain in weight at 6° averaged 17.3 per cent; at the lower temperature, only 4.5 per cent. This insect is protected by the action of the low temperature from excessive hydration and is prevented from becoming too susceptible to cold. Similar results were obtained with hibernating *Polistes variatus* queens and with dormant prepupae of *Lucilia sericata*. In every case the return to normal activity was hastened by a gain in water.

FACTORS WHICH INFLUENCE SURVIVAL AT LOW HUMIDITIES

Binding of water by hydrophilic colloids.—Robinson (1926) demonstrated that granary insects, which live in relatively dry environments and which eat food which is low in water (9–11 per cent), also have a low water content, this value for the granary weevil, *Sitophilus granarius*, being 46–47 per cent, and for the rice weevil, *Sitophilus oryza*, 48–50 per cent. Robinson made determinations of the free and bound water present in these insects, using the method of Thoenes (see Gortner, 1938, p. 285). The granary weevil had 50 per cent of its total water bound by the body colloids, while the rice weevil had 35 per cent of its water in the bound condition. On the other hand, those forms which live on very moist foods have a high water content, only from 3 to 9 per cent of which is bound. Since water in combination with hydrophilic colloids cannot be removed readily by desiccation, the binding of a large amount of water in granary insects is a process which conserves water and permits these forms to live under low moisture conditions.

Conservation of metabolic water.—Many insects are able to survive prolonged exposures to dry atmospheres by the conservation of water resulting from the oxidation of foods. In most organisms much of this water is excreted, but in many insects it is used over again in metabolism. These insects may be desiccated for a long time before any change in relative water content is noted. Thus, Berger (1907) showed that when the meal worm *Tenebrio molitor* is kept in a dry medium, in spite of a great loss of weight, the relative fluid content of its body remained approximately constant. Hall (1922) found that the meal worm can be desiccated to 52.6 per cent of its original weight without loss of vitality; and Buxton (1930) showed that this insect can maintain the normal proportion of water in its body during a month's fast at humidities from 0 to 60 per cent. Mellanby (1932*a*) made a study of the metabolism of the fasting meal worm and demonstrated that the loss of solid matter during starvation was approximately balanced by the production of metabolic water. According to Mellanby's calculations, when 0.624 gm. of solids are lost, 0.615 gm. of water are produced. When meal worms are starved at different humidities, they retain their

original ratio of water to dry matter; but they lose weight at a different rate at each humidity, this loss of weight being roughly proportional to the saturation deficiency of the atmosphere. Hence the rate of metabolism is different at each humidity, and it is regulated so as to produce sufficient water of metabolism to maintain a constant percentage of body fluid. Another insect able to maintain a constant percentage of body water when exposed to dry air is the Mediterranean flour moth, *Ephestia kuehniella*. Speicher (1931) placed the second and third larval instars of this insect in jars containing anhydrous $CaCl_2$ and fed them meal which had been previously dried at 103° C. As long as they survived, they maintained a constant percentage of body water (65.5 per cent). Robinson (1928) also demonstrated that the conservation of metabolic water is an important factor in the survival of granary insects at low atmospheric humidities.

Many insects possess a more limited power to conserve metabolic water. Adult bedbugs (*Cimex lectularius*), starved at different relative humidities, lost weight more rapidly in dry than in moist air but consumed the same amount of dry matter in each case, thus indicating a more rapid loss of water in dry air (Mellanby, 1932*b*). However, when saturation deficiencies were compared, the loss was relatively greater in moist air; but the surface area of these small insects is so great in comparison with their volume that they cannot prevent the loss of a large proportion of the body water. Buxton (1932*b*) placed unfed adults of the bloodsucking bug *Rhodnius prolixus* at 23° C. and at relative humidities of 0, 30, 60, and 90 per cent. The loss of weight at 90, 60, and 30 per cent was not affected by humidity even during a fast of 42 days. However, bugs kept in drier air lost more water and oxidized less solids than those kept in moist air, so that the proportion of solid matter increased in the drier atmosphere. The conservation of metabolic water seems unlikely, although the insect possesses considerable powers of regulating the proportion of water in its body. This mechanism was found to break down after 21 days at 0–30 per cent and after 42 days at 60 per cent humidity. Ludwig (1937) starved the nymphs and adults of the grasshopper *Chortophaga viridifasciata* at different relative humidities and a constant temperature of 25° C. These insects possess the ability to conserve metabolic water at relative humidities down to 12 per cent. However, this ability did not persist in insects exposed to dry air, where the loss of weight was almost entirely due to the loss of water. Hall (1922) studied the rate of exsiccation of many kinds of animals, including several species of lizards and turtles. These forms are able to conserve water by the excretion of nitrogenous wastes in the form of uric acid instead of urea. As a result, they are able to withstand a longer period of desiccation than forms which do not produce uric acid. Wigglesworth (1939) stated that all of the insects studied excrete nitrogenous wastes in the form of uric acid, thus conserving water for further use in metabolic activity.

In general, organisms which are not normally exposed to low humidities do not possess the power of water conservation. MacLeod (1935) observed that the tick *Ixodes ricinus* is not able to conserve water, the loss of weight at low humidities being a direct indication of the evaporation of water from the body. Similar observations were made by Evans (1935) for the prepupae and pupae of the blowfly *Lucilia sericata;* and by Ludwig and Landsman (1937) for the larvae, prepupae, and pupae of the Japanese beetle, *Popillia japonica*.

Initial water content.—It appears probable that an organism with a high water content may be able to survive longer periods of exposure to dry air than one with a low water content. There is very little experimental evidence available to test the validity of this assumption. Ludwig (1937) starved hibernating nymphs of the grasshopper *Chortophaga viridifasciata* with a low average water content of 65.6 per cent, and growing nymphs and adults of the same species with an average water content of 75.9 per cent, in dry air at 25° C. and determined the rate of desiccation, the water content, and the percentage of weight loss for each group. The fatal limit of desiccation was approximately the same for each group (56.1 and 58.8 per cent water), although the hibernating nymphs had lost only 27.5 per cent of their original weight, while this value for the growing nymphs and adults was 44.8 per cent. It is interesting to note that in this experiment the rate of water loss was the same for each group and that those with the low initial water content survived less than 2 days, while those with the high initial water content survived more than 6 days (unpublished data). This inability of hibernating forms to survive prolonged exposures to low humidities is interesting in view of the findings of Hodson (1937) that most insects about to enter hibernation seek moist environments.

Ludwig (1942) exposed the eggs of the Luna moth, *Tropaea luna*, to different relative humidities at a temperature of 25° C. and found that a high percentage (26.3–45.8 per cent in different lots) produced normal larvae in dry air. In 1943 he repeated the experiment, using the eggs of the Cynthia moth, *Samia walkeri*, and did not obtain larvae at humidities below 16 per cent. A comparison of the initial water contents of the eggs of the two species showed that these values for the newly laid Luna eggs averaged 67.4 ± 0.008 per cent, whereas for the Cynthia eggs they averaged 61.9 ± 0.028 per cent. It appears likely that, because of their low initial water content, the Cynthia eggs became desiccated beyond their vital limit at low humidities, before the larvae were ready to hatch.

Rate of desiccation.—There is a considerable amount of experimental evidence which indicates that a rapid loss of water is more injurious than a more gradual desiccation. The rate of evaporation is dependent upon the relative amount of surface per unit volume of the organism and on the nature of the surface. These factors are important in determining the ability of many organisms to withstand exposures to low humidities.

Durig (1901) found that rapid desiccation resulted in the death of the European frog when only 15 per cent of the frog's weight was lost. However, if the drying was slow, the frog could lose 30–39 per cent of the body weight before dying. Gunn (1933) observed that the cockroach *Blatta orientalis* survived a weight loss of 44 per cent during slow, and only 32 per cent during rapid, desiccation. Shelford (1913) performed desiccation experiments on a number of insects and amphibians. He made the statement that "animals die after a smaller amount of evaporation when the rate is slow than when it is more rapid." However, in view of the other experiments cited in the present discussion, this conclusion appears questionable. Shelford noted that the smaller animals died from loss of water much more quickly than the larger ones. He associated this difference with the relatively greater surface area of the smaller animals. The greater surface area resulted in a more rapid loss of water, and hence the animals reached the vital limit of desiccation in a shorter time.

Talbot (1934) subjected a number of species of ants of the genera *Formica* and *Lasius* to controlled conditions of dryness. In all of the species tested the females lived longer than did the workers or males of the same species. This greater ability to survive at low humidities is due, at least in part, to the slower rate of water loss associated with the larger size of the females. Gunn (1935) compared rates of weight loss in dry air of three species of cockroaches: *Blatella germanica*, *Blatta orientalis*, and *Periplaneta americana*. *Blatella germanica*, the smallest species, lost weight most rapidly; and *P. americana*, the largest species, most slowly. During desiccation of the Japanese beetle, *Popillia japonica*, in its various developmental stages (Ludwig, 1936) the first-instar larvae lost water at a more rapid rate than the other stages, presumably because of its relatively larger surface area. Rapid desiccation of third-instar larvae was found to be more harmful than slow desiccation. In the former case the fatal limit was a loss of 46 per cent of the original weight; while in the latter case this value was 50 per cent. The ability to survive a greater degree of desiccation when the rate of evaporation is slow is probably an adaptation enabling the insect to survive periods of drought under field conditions.

Ludwig and Anderson (1942) made an analysis of the various factors associated with the survival of the eggs of saturniid moths under different temperature and humidity conditions. Rates of dehydration were correlated with the size and surface area of the eggs. The eggs of the Promethea moth, *Callosamia promethea*, and of the Cynthia moth, *Samia walkeri*, lose water more rapidly than those of the Cecropia moth, *Platysamia cecropia*, at comparable temperatures and humidities. The eggs of the first two species are much smaller than those of the latter species, weighing 2.0 mg. each, as compared with 4.5 mg. Hence, their more rapid rate of desiccation is associated with their relatively greater surface area. Another interesting comparison, associated with surface area, concerns the more rapid rate of dehydration of eggs of the Polyphemus moth, *Telea polyphemus*, as compared with those of the Cecropia moth. These two species of moths lay eggs of approximately the same size. However, the shapes of the eggs are different. The Polyphemus egg is discoidal, and therefore its surface area per unit weight is greater than that of the more nearly ovoid egg of the other species. Hence, it appears that the rate of dehydration under comparable conditions and the ability to survive at low humidity are associated with the size and the relative amount of surface area of the organism. The larger animal, because of its slower rate of water loss, is better able to survive exposures to low atmospheric humidities than is the smaller one.

Ability to survive the loss of large amounts of water.—Animals show a considerable variation in the amount of water which they can lose and still retain their vitality. In many organisms this vital limit determines the ability to survive under conditions of low atmospheric humidities. Breitenbrecher (1918) determined the vital limit of desiccation for several species of beetles. The June beetle *Lachnosterna* could survive a loss of 15 per cent of its original weight; whereas *Cotalpa lanigera* could survive a loss of 25 per cent, and the potato beetle, *Leptinotarsa decemlineata*, a loss of more than 50 per cent. Breitenbrecher found that when the potato beetle was introduced from its grassland habitat into an arid region it became equilibrated immediately with respect to its surroundings, especially in regard to its water and temperature medium. Its be-

havior also became modified to resemble the responses of those organisms long accustomed to a desert complex. The ability of this insect to adapt itself to arid conditions is undoubtedly associated with its ability to survive a high degree of desiccation. Buxton (1932*b*) found that the bug *Rhodnius prolixus* could tolerate a very great loss of weight—in one experiment, for example, almost 50 per cent of its original weight after starvation for 42 days. At the lower humidities this weight loss was due largely to a loss of water. Ludwig (1936) showed that first- and third-instar Japanese-beetle larvae survived a loss of 50 per cent of the original weight when the rate of desiccation was slow. Second-instar larvae were more resistant, surviving a loss to 45 per cent of the original weight.

Hall (1922) observed enormous differences in the amount of water which various animals can lose by desiccation without a loss of vitality. In general, the less complex animals can withstand a greater water loss. The earthworm *Allolobophora chloroticus* can lose 69.6 per cent of its body weight, or approximately 83 per cent of the water contained in its body, without loss of vitality. The meal worm *Tenebrio molitor* was desiccated to a loss of 52.6 per cent of its weight. In the latter case the weight loss was greater than the weight of the water contained in its body before desiccation. As explained above, the meal worm is able to conserve metabolic water; and, when desiccated, it loses water and dry materials at the same rate, thus maintaining a constant proportion of water as long as it remains alive. The frog *Rana pipiens* survived a weight loss of 41.0 per cent, and the salamander *Amblystoma punctata* of 47 per cent, of the original weight. Hall found that the horned "toad" *Phrynosoma cornutum* and the desert lizard *Sceloporus spinosus* are very resistant to desiccation. They were able to live in dry air, without food or water, apparently with no discomfort, for nearly 4 months. The horned "toad" resisted desiccation to a loss of 33.8 per cent; the desert lizard, to 47.8 per cent of the original weight. Other reptiles were also very resistant to loss of water. The turtle *Chrysemys marginata* survived a loss of 33.1 per cent of body weight; the chameleon *Anolis carolinensis*, of 46.3 per cent. The time of survival was very much reduced in the last two, as compared with the first two, species of reptiles. The figures given by Hall represent the maximum amount of weight which could be lost by the species studied. The figure given for the frog is slightly higher than that (30–39 per cent of body weight) obtained by Durig (1901) for the European frog during slow desiccation.

Thorson and Svihla (1943) compared the vital limits of desiccation of different species of amphibians with the type of environment in which they normally live. In their experiments the frog *Rana pipiens* survived an average loss of 35.5 per cent of body weight. This value is in agreement with the figure given by Durig. Those amphibia which are able to live in relatively dry environments can survive the greatest water loss. The toads *Scaphiophes holbrookii* and *S. hammondii*, which live in semiarid regions, survived water losses of 47.9 and 47.6 per cent of body weight, respectively, while the terrestrial toads *Bufo borealis* and *B. terrestris* survived water losses of 44.6 and 43.3 per cent of body weight. The various species of *Rana*, which are semiaquatic, survived water losses of 29.5 to 35.5 per cent of body weight. All species studied were found to be highly susceptible to desiccation. Hence, Thorson and Svihla conclude that, among amphibians at least, the transition to terrestrial life has been made possible,

to a great extent, not by the development of resistance to evaporation but by the gradual evolution of the ability to lose a large percentage of body water and yet survive.

Presence of an impermeable covering.—The ability of many organisms to survive low humidities depends upon the presence of a body covering which is relatively impermeable to water. The importance of the chorion in preventing excessive loss of moisture from the insect egg at low humidities has already been discussed. However, several investigators have reported permeability changes in the membranes of insect eggs which affect resistance to desiccation. Thompson and Bodine (1936), using the egg of the grasshopper *Melanoplus differentialis*, and Birch and Andrewartha (1942), using the egg of the grasshopper *Austroicetes cruciata*, reported variations in the ability of the eggs to resist desiccation in different developmental stages. Prediapause eggs were very susceptible to desiccation, whereas diapause eggs were more resistant. This difference in susceptibility to water loss was correlated, by Birch and Andrewartha, with the absence of membranes, which they called the yellow and white cuticle, in the prediapause eggs and their presence in the diapause eggs. Postdiapause eggs were most easily desiccated. The difference between diapause and postdiapause eggs could not be correlated with any detectable change in the morphology of the egg membranes. However, since the emergence from the diapause condition is associated with the absorption of water, the egg membranes were stretched considerably thinner in the postdiapause eggs. Thompson and Bodine reported that in the postdiapause egg, the chorion was also cracked, sometimes completely peeling off the egg. They believed, however, that the increased permeability of the egg membranes is associated with the thinness of the cuticle and not with the peeling of the chorion.

Buxton (1932*a*) gave a comprehensive discussion of the various sites of water loss from the body of an insect. A considerable amount of water is undoubtedly lost through the alimentary canal in those homopteran insects which feed on plant sap and which possess filter chambers to facilitate the quick passage of liquids and at the same time the concentration of nitrogenous compounds. In other forms, such as the meal worm, the amount of liquid lost is very small, the rectum and hindgut being very efficient in the conservation of water. Furthermore, the Malpighian tubes assist in this conservation by the production of uric acid. Wigglesworth (1931*a*) found that the lower part of the Malpighian tubule functions in the reabsorption of water, and in another publication (1931*b*) he suggested that reabsorption of water from the excrement occurs through the rectal glands and the rectal epithelium. Wigglesworth (1931*c*) observed that the tracheoles of insects, both aquatic and terrestrial, contain a fluid, the amount of which is controlled by the osmotic pressure of the body fluids. Buxton (1932*a*) believed that the loss of water from the insect's body occurs largely through the tracheal system. Mellanby (1934) performed some experiments to determine whether the water evaporated from an insect's body in dry air is lost through the spiracles. He used the larvae of both the meal worm *Tenebrio molitor* and the clothes moth *Tineola bisselliella* and the larvae and adults of the flea *Xenopsylla cheopis* and compared the rate of water loss of normal insects with that of others kept in gas mixtures (such as air plus 5 per cent of carbon dioxide) that caused the insects to keep their spiracles permanently open. Insects

treated with these gas mixtures lost water from two to seven times as fast as untreated ones. He concluded that practically all of the water evaporated from the insect is lost by way of the tracheal system and that a thin integument may be just as watertight as one which is highly sclerotized. Manton and Ramsay (1937), in comparing rates of water loss, found that the onychophoran *Peripatopsis* lost water twice as fast as the earthworm, forty times as fast as a smooth-skinned caterpillar, and eighty times as fast as a cockroach. In *Peripatopsis* water loss is very rapid, probably because it occurs through the spiracles, which are numerous and have no closing mechanism. As will be discussed later, certain insects control the amount of water lost through the tracheal system by keeping their spiracles closed when exposed to low humidities. Davies (1928) has shown that nontracheate species of Collembola rapidly become desiccated at 25° C. and at the humidity of 30 per cent; individuals of *Isotoma viridis* die within ½ hour. The loss of water from this species must occur through the general body surface.

Gunn (1933) studied the evaporation of water from the cockroach *Blatta orientalis*, and Ramsay (1935*b*) performed similar studies on the cockroach *Periplaneta americana*. Ramsay found that water loss occurred through both the tracheal system and the general body surface. Both authors reported that above 30° C. the rate of evaporation increased enormously with increase in temperature. Gunn believed that this greater loss of water was due to an increase in respiratory movements by which water was swept out of the tracheal system. However, Ramsay obtained similar results when the spiracles were blocked with wax. He suggested that 30° C. may be connected with the melting of a fat, possibly a fatty substance covering the cuticle of the insect. His observations indicated the presence of a waxy film on the surface of the insect at temperatures below 33° C. but its absence at higher temperatures. The presence of this film undoubtedly serves to make the cuticle more impermeable to water at temperatures below its melting-point. Ramsay's experiments show that the generalization advanced by Mellanby (1934), that the tracheal system is the site of water loss from insects, is unsound.

Hall (1922), as a result of his studies on the desiccation of a number of animals, concluded that the length of time an animal can endure atmospheres of low humidity depends primarily upon the type of integument and secondarily on the proportion of body surface to body mass. Gray (1928) compared the rate of desiccation of the newt *Triton cristatus* with that of the lizard *Lacerta viridis*. The newt lost water very rapidly when exposed to the air, and it absorbed water readily when placed in an aquatic medium. Furthermore, living and dead newts lost water at the same rate in air. On the other hand, the lizard lost water very slowly when exposed to air. This ability to conserve water was located in the skin, since lizards with their skins removed lost water as rapidly as newts. Gray concluded that the origin of terrestrial vertebrates is associated with the development of a skin which is relatively impermeable to water. Hall and Root (1930) pointed out that the possession of a scaled integument in the reptiles enabled them to resist desiccation for long periods of time. Thorson and Svihla (1943), in their comparison of the resistance of frogs and toads to desiccation, also state that a difference in the permeability of the skins of the two groups of amphibians assists the latter group to resist desiccation but is not the primary factor. Adolph (1933) reported that toads are

not able to absorb water from a saturated atmosphere but that they can take up contact moisture. He believes that their ability to survive away from water is due to their ability to absorb water from moist soil.

Howes and Wells (1934*a*, *b*) compared the water relations of terrestrial snails and slugs, using specimens of *Helix pomatia*, *Arion ater*, and *Limax flavus*. When the snail is desiccated, it retires into its shell, loses weight extremely slowly, and can survive without water for over 10 months. However, the slugs lose water very rapidly at humidities between 40 and 80 per cent (temperatures between 15° and 19° C.) and die before the end of the second day. Hence, the slug must live in a moist environment because it possesses a skin which is freely permeable to water.

The above discussion indicates that in many diverse groups of animals the character of the body covering determines, to a certain extent, the ability of the animal to survive in relatively dry environments. Some animals, such as amphibians and slugs, with freely permeable skins, must remain in contact with moisture if they are to survive. Others, like insects and lizards, have developed a body covering which is relatively impermeable to water and which permits the animal to live independently of contact moisture. In the case of certain insects, the water permeability of the body covering is decreased by the presence of a waxy film, with phase and associated permeability changes at approximately 30° C.

Rate of metabolism and rate of development.—Many organisms are able to survive prolonged exposures to low humidities because partial desiccation slows down the metabolic rate, with the result that the rate of water production from metabolism is considerably reduced. Hall (1922) gave a review of the literature on desiccation and pointed out that "some animals become immobile when gradually dried out, and are apparently dead." However, in this state they may be able to survive long periods without food and water. Wodsedalek (1912) reported keeping the larvae of the dermestid beetle *Trogoderma tarsale* in vials in the laboratory without food or water for over a year. During this time they molted, becoming smaller after each molt. Hall associated this ability to survive without food and water with a reduced metabolic rate. Buxton (1930), Mellanby (1932*a*, *b*), Gunn (1933), Thompson and Bodine (1936), and Ludwig (1936, 1937) observed that when insects are exposed to low humidities they lose weight rapidly at the beginning of the desiccation period and the rate of water loss progressively decreases. Mellanby believed that the progressive decrease in water loss may be due to a decline in metabolic rate as desiccation proceeds. Ludwig observed that the activity of Japanese beetle larvae decreased during desiccation and that they were practically quiescent by the end of the first day. This quiescent condition was associated with a diminishing metabolic rate. As further evidence, he cited the relatively slow rate of desiccation of the prepupal and pupal stages, which are also characterized by a decrease in metabolic rate, as indicated by their low rate of respiratory exchange (Ludwig, 1931). Thompson and Bodine (1936) associated the slow rate of desiccation of the diapause egg of the grasshopper with a low rate of metabolic activity. Buxton (1932*b*) believed that the ability of *Rhodnius prolixus* to survive prolonged exposures to low humidities was correlated with reduced rate of metabolism. He postulated that the effect of dry air on this insect is to keep the spiracles closed and

that hence there is a reduction of rate of dehydration and also of metabolism, so that less solids are used in dry than in moist air. The increased proportion of solids increases viscosity and slows down the rate of diffusion.

Howes and Wells (1934*a*) found that dehydration of the snail *Helix pomatia* caused it to aestivate. In these animals aestivation is always the result of low water content. It may be brought about either by dryness of the environment or by the natural dehydration cycle of the animal. During aestivation the metabolic rate is probably reduced to a low level.

The rate of development of an insect may also be a factor influencing its ability to survive desiccation. Evans (1936) compared the humidity requirements of several species of blowflies. The eggs of *Calliphora erythrocephala* and *Phormia groenlandica* showed a higher mortality than those of *Lucilia sericata* when exposed to low humidities. The higher mortality of the first two species was brought about by their slower rate of development and consequent longer exposure to the desiccating effects of dry air. Ludwig and Anderson (1942) made comparable observations with the eggs of several species of saturniid moths. The eggs of the Polyphemus moth, *Telea polyphemus*, were able to develop and to hatch over a wider range of temperature and humidity combinations than the eggs of the other species of saturniid moths studied. Polyphemus eggs also showed a more rapid development, particularly at the higher temperatures. This rapid developmental rate permitted the larvae to complete their embryogeny and to hatch over a wide range of humidities, because they were ready to hatch before they became excessively desiccated.

A reduced rate of metabolism is an important factor in the survival of certain animals at low humidities because less solids are used, with the result that less water is made available for evaporation. Many animals become quiescent when exposed to dry air. Conversely, a rapid rate of development may be advantageous because the animal may complete its development at a low humidity before it becomes dehydrated beyond its vital limit.

Selection of favorable environments.—Thus far, the mechanisms which determine the survival of organisms when they are confined to low humidities and constant temperatures have been discussed. These mechanisms consist very largely of metabolic changes which result in the conservation of water by the organism. However, under field conditions the animal may choose an environment which assists it in surviving unfavorable humidity conditions. Many investigators have found that low humidity modifies the behavior of an organism and that, in many cases, this changed behavior pattern favors survival. Bodenheimer (1931) experimented with the adults of the beetle *Adesmia clathrata* and found that the temperature preference was not affected by humidity. However, if the insects were first conditioned by exposure for 1 to 2 weeks at various controlled humidities, their preferred temperatures were very different. Those kept previously at 20 per cent humidity preferred 36.6° C., and those kept at 90 per cent preferred 39.4° C. The lower temperature tends to decrease the evaporation of water. Gunn (1931) experimented with the cockroach *Blatta orientalis* and observed that when individuals are more dehydrated they tend to select lower temperatures. In 1934 he showed that desiccating the cockroach to 70 per cent of its original weight caused it to prefer a lower temperature.

The preferred temperature range of the normal adult male is from 20° to 29° C.; that of desiccated specimens, from 12° to 23° C. After the insects had been allowed to drink, the preference returned to normal temperature. On the other hand, Bodenheimer (1931) found that tenebrionid beetles *Zophosis punctata* conditioned at low humidities selected higher temperatures than those conditioned at higher humidities. Gunn (1934) questioned the methods used in the experiments and the validity of the conclusions. Gunn and Cosway (1938) tested the preferred temperatures of cockroaches without previous desiccation. They found a slight but significant difference when individuals were tested in dry and in moist air, those in dry air preferrring a slightly lower temperature.

The fact that many insects seek a moist environment for hibernation has already been mentioned. The literature contains a number of references to the positive responses of organisms to water or to moist air. Gunn and Kennedy (1936) devised an apparatus by which they were able to maintain a straight-line gradient of humidity in a closed channel and by means of which they could present any two humidities to an animal as alternatives and study its reactions. In 1937 Gunn studied the reactions of the isopod crustacean *Porcellio scaber* to humidity. This organism is very active in dry air and nearly motionless in saturated air. Individuals were found to congregate in moist air. Waloff (1941) observed the humidity reactions of three species of terrestrial isopods—*Oniscus asellus*, *Porcellio scaber*, and *Armadillidium vulgare*—verifying the observations of Gunn. All three species congregated in moist air. In each species there was a decrease in activity in moist air, but the animals turned around frequently, with the result that they remained in areas of greater humidity. Gunn and Cosway (1938), experimenting with the cockroach *Blatta orientalis*, obtained a mixed reaction when normal individuals were exposed to a humidity gradient. However, on desiccation there was found to be a tendency for them to become hygropositive. Kennedy (1937) showed that the grasshopper *Locusta migratoria migratorioides* preferred dry air, although it was by no means optimal for development, maturation, or breeding. Keeping the organisms in dry cages for a few days did not destroy the dry-air preference. Pielou and Gunn (1940) reported that normal specimens of adult *Tenebrio molitor* tend to congregate in drier air. However, when the highest humidity was not above 70 per cent, only a slight reaction was obtained. Lees (1943) found that the larvae of the click beetle *Agriotes* avoid dry air, the intensity of the reaction being greatest when the alternatives are close to saturation. Thus, when alternate humidities were 99.5 and 92 per cent, all of the larvae were recovered from the wet side of the chamber. However, when the alternatives were lower on the humidity scale, fewer larvae gave the hygropositive reaction. The reaction was in better accord with humidity differences when these are measured as saturation deficiencies rather than as relative humidities. The humidity receptors are located on the head region of the insect, distributed among the antennae, and the maxillary and labial palps. Bentley (1944) observed that the beetle *Ptinus tectus* tends to congregate in dry air, remaining for a period of 5 or 6 days, after which they disperse and migrate up the humidity gradient. After 8 days, 94 per cent of the beetles were in the immediate vicinity of water. In this species the humidity receptors are located on the antennae. The reaction is not equally apparent over all humidity ranges and is

usually absent between 75 and 90 per cent relative humidity.

In some of the above experiments the organisms were brought into environments which are not optimal for development or survival. However, desiccation usually resulted in a weakened or a reversed reaction. This modification of the response is undoubtedly a protection against prolonged exposures to dry atmospheres. Those organisms which normally live in very moist environments, when given a choice, show a preference for moist air; and those which live in relatively dry environments, such as granary insects and insects which feed on stored products, show a preference for dry air. These reactions may be considered adaptive because they tend to bring the organism into an environment best suited for its particular type of metabolism.

RELATIVE HUMIDITY AND DISTRIBUTION

From the preceding discussion it is evident that atmospheric humidity exerts a controlling influence on the distribution of organisms. Obviously, those forms which possess no methods of conserving water and are not protected from water loss by the presence of an impermeable body covering must live in an environment with a high moisture content. Thus, Friedel (1928) reported that humidity, water content of soil, and dampness of soil air are the most important factors in the distribution of the symphylid *Scutigerella immaculata*. MacLeod (1936) observed that a wet, mossy, or peat soil and a dense mat of old vegetation or a rank growth is necessary for the survival of the tick *Ixodes ricinus*. In Britain the critical season for the survival of this species is the summer, during which the moisture factor in the microclimate acts in a limiting capacity. Howes and Wells (1934*b*) demonstrated that the slugs *Arion ater* and *Limax flavus* lose water very rapidly under laboratory conditions and must drink water periodically if they are to survive. Similarly, Durig (1901) and Adolph (1933) observed that amphibians lose water even in saturated air and must be in contact with moisture if they are to maintain a constant water content. On the other hand, many organisms possess means of preventing loss of moisture and can survive in relatively dry environments for long periods of time. Granary insects and those which feed on stored products are examples of this type.

Among closely related species there exist wide variations in moisture requirements and in habitats. Rubtzov (1934) divided Siberian grasshoppers into four main classes, depending on the humidity conditions of the habitats in which they occur. These classes are hygrophile, mesophile, xérophile, and extreme xerophile. Although xerophilic organisms must be better able to withstand exposures to low humidities than hygrophilic forms, very little experimental evidence to substantiate this belief was available until recent years. Talbot (1934) and Gösswald (1938) studied the moisture requirements of ecologically different species of ants. Talbot compared species found in open dunes, fields, or prairies, where summer draughts are often severe (*Formica truncicola obscuriventris*, *F. pallide-fulva schaufussi incerta*, and *F. cinerea neocinerea*), with other species abundant in regions where the water supply is more constant (*F. fusca subsericea*, *F. sanguinea rubicunda*, and *F. ulkei*). The former group was able to withstand dryness at higher temperatures than the latter group. No such differentiation could be found for species of *Lasius*. These ants do not forage on hot days, as do species of *Formica*. Hence,

the physiological differences observed appear to be definitely adaptive, in that they are correlated with the type of habitat the animal can utilize. Gösswald compared the temperature and humidity requirements of hygrophilic ants (*Tetramorium caespitum* and *Lasius niger*) with xerophilic species (*Leptothorax nigriceps* and *L. unifasciatus*). The former group was able to survive much longer at relative humidities of 95–100 per cent (temperature, 15° C.) than at lower humidities. At humidities from 35 to 90 per cent very little difference in the time of survival was noted. The latter group survived much longer at low humidities than the former group. Here, also, physiological differences are adaptive and correlated with the humidity conditions of the habitats in which the different species occur.

Kofoid (1934) and Williams (1934) studied the factors determining the distribution of different species of termites. Kofoid postulated that some termites possess the ability to create and maintain a high humidity in their burrows with the aid of moisture of metabolism, of wood and soil; thus, dry-wood termites are able to invade dry wood independently of ground connections and to live in semiarid and even desert regions. Some species of *Amitermes* build earthen galleries around wooden posts, desert plants, etc., and thus actually live in a saturated atmosphere. According to Williams, moisture is by far the most important single factor in the ecology of *Reticulitermes hesperus* and *R. tibialis*. Both species, being subterranean, require a constant supply of water in soil, in food, and in the air. Although they are not able to withstand desiccation, they may extend their activities above ground by building covered runways, thus extending subsoil conditions for short distances above ground. When an increase in temperature occurs, relative humidity is lowered, and the termites migrate downward. *Reticulitermes tibialis* is characteristic of more arid regions and can withstand smaller amounts of moisture than *R. hesperus*. Williams tested the behavior of various species of termites in a humidity gradient. Most of the species studied preferred 100 per cent; but *Kalotermes minor* and *K. hubbardi* showed a marked indifference to humidity. They are dry-wood forms and frequently live in a relatively dry atmosphere.

Geisthardt (1937) compared the humidity requirements of two species of bedbugs—*Cimex rotunda*, a tropical species, and *C. lectularius*, which is rather widely distributed throughout the world. *Cimex rotunda* has its optimal humidity at 93 per cent (temperature, 27° C.), while for *C. lectularius* it was 75 per cent. Furthermore, *C. rotunda* was greatly affected by low humidities, development at 6 per cent being retarded at all temperatures; whereas, at this humidity, development of *C. lectularius* was retarded only at the extremes of temperature. The latter species is adapted by physiological reactions to its wider distribution.

Ludwig (1942, 1943) compared the humidity requirements of pupae of the Luna moth, *Tropaea luna*, and the Cynthia moth, *Samia walkeri*. The pupal stage of the Luna moth is passed within a cocoon formed on the ground among the leaves of the larval food plant, where it remains throughout the winter under relatively moist conditions. On the other hand, the cocoons of the Cynthia moth are suspended from the branches of trees, where the pupae are subjected throughout the winter to the drying effects of the atmosphere. Cynthia pupae, in comparable physiological stages and under the same humidity conditions, are more resistant to desiccation than Luna pupae. Furthermore, Cynthia pupae can be

desiccated to a greater degree before there is a decrease in the egg production of moths which develop from the dehydrated pupae. They are thus better adapted to overwintering at low humidities than are the pupae of the Luna moth.

Similar observations have been made on a few species of amphibians and reptiles. Shelford (1913) observed that the salamander *Plethodon glutinosus*, which lives in damper regions, is more sensitive to dry air than is *P. cinereus*. Hall (1922) found that the horned "toad" and the desert lizard, which live in very dry habitats, are very resistant to desiccation. Gray (1928) pointed out that terrestrial vertebrates evolved without a reduction in the percentage of body water but with the development of a relatively impermeable skin. Thorson and Svihla (1943) demonstrated that terrestrial amphibians are able to survive the loss of large amounts of water.

SUMMARY

1. Atmospheric humidity affects the organism by modifying its water content. Some animals are hygroscopic and absorb water from moist air. Others lose water at all humidities, including saturated air. In general, the rate of water loss is greater, the lower the humidity.

2. The moisture-holding capacity of the air is greater at higher temperatures, and hence relative humidity at different temperatures indicates very different moisture conditions. For this reason the saturation deficiency of the air is considered a more reliable index of the evaporating power of the air. However, the loss of water from organisms at the same saturation deficiency is usually greater at high than at low temperatures. High temperature may bring about this effect by (*a*) accelerating the rate of metabolism, with the result that metabolic water is produced and lost at a greater rate; (*b*) increasing the rate of diffusion, thus increasing the rate of evaporation; and (*c*) increasing the water permeability of the body surface.

3. The optimal humidity may be defined as (*a*) the humidity at which development occurs most rapidly; (*b*) the humidity which permits the greatest number of individuals to complete development; (*c*) the humidity at which the reproductive capacity of the species is greatest, i.e., the largest number of progeny per female is produced; and (*d*) the humidity which permits survival over the widest range of temperature conditions. For most species the optimal humidities are in the upper part of the humidity range but below the saturation-point. Saturated air is usually unfavorable because it favors the growth of parasitic fungi. Low humidities are unfavorable because the organism may become dehydrated beyond the vital limit before it is able to complete its development. Many insect eggs are protected from excessive desiccation by the presence of a relatively impermeable chorion. However, at low humidities the larvae may not be able to hatch because they are killed by desiccation after having perforated the chorion. In other cases the chorion itself becomes too hard from water loss to permit hatching.

4. The effect of humidity on the developmental rate of an insect is modified by temperature in the following ways: (*a*) in a few insects the developmental rate is independent of humidity at all temperatures which permit development; (*b*) in some species it is independent of humidity only in a particular temperature range; and (*c*) in others development is retarded at low humidities regardless of temperature. For every favorable temperature there is a definite range

of humidities in which it is possible for development to occur. Usually this range is widest at those temperatures which permit the highest percentage of survivals.

5. The optimal humidity for development or for survival may shift with temperature.

6. Relative humidity assists in regulating the body temperature of an animal through its control of water loss. In dry air many insects have body temperatures which are lower than environmental temperatures because of the cooling effect of evaporation. This action is an important factor in the survival of the insect at high temperatures. Amphibians and reptiles also have body temperatures lower than those of the environment at low humidities.

7. Relative humidity modifies the thermal death-point of insects in the following ways: (*a*) some insects are killed at the same temperature regardless of humidity; (*b*) certain insects lose so much water in dry air that the fatal temperature is lower (as a result of desiccation) in dry than in moist air; and (*c*) some insects are capable of surviving a higher temperature in dry than in moist air, owing to the lowering of the body temperature by evaporation.

8. Relative humidity modifies the survival of organisms at low temperatures by controlling the amount of body water. Any process which reduces the amount of freezable water in an insect increases its cold-hardiness, provided this reduction is not excessive. During exposures to low temperatures a moist environment is beneficial because the latent heat of water prevents a very rapid drop in temperature. The organism is prevented from hydration under such conditions because the low temperature prevents the absorption of water by the body.

9. Many factors are concerned with the ability of organisms to survive exposures to low humidities.

a) Some insects are able to live in relatively dry environments and eat foods with a low water content because a high percentage of their total water is held in the body colloids and cannot be readily removed by desiccation.

b) Many insects conserve and use the water produced by the oxidation of foods. They may be desiccated for prolonged periods of time before any change in the relative water content is noted. Other insects possess a more limited power of conserving metabolic water. In some cases this mechanism is effective at moderately low humidities but breaks down during exposures to dry air.

c) Members of the same, or closely related, species having a high initial water content can survive longer periods of exposure to dry air than those with a low initial water content. The fatal limit of desiccation is apparently not modified by the initial percentage of water.

d) A rapid loss of water is more injurious than a gradual desiccation. The rate of evaporation is, in turn, dependent on the relative amount of surface per unit volume and on the nature of the body surface. In general, because of their relatively smaller surface areas, larger organisms are able to survive longer exposures to low humidities than smaller ones. The slow rate of dehydration which occurs under field conditions enables the animal to survive periods of deficient rainfall.

e) In general, those forms which live in dry environments are able to survive because they can withstand the loss of relatively larger amounts of water.

f) Variations in the ability of organisms to resist desiccation during different stages of development have been correlated with changes in the water permeability of the body covering. Different

species of insects vary greatly in their ability to resist desiccation. Many insects lose water through their tracheal systems. When exposed to low humidities, these insects may decrease the rate of water loss by closing their spiracles. Variations in the ability of different species to withstand desiccation may also be associated with the permeability of the body covering. Lizards with their skins removed lose water at the same rate as the intact frog.

g) Rate of metabolism may also be correlated with survival at low humidities. Partial desiccation slows down metabolic rate and, hence, the production of metabolic water. Many organisms can survive prolonged periods of exposure to low humidities in a partially desiccated condition. In some cases, partial desiccation induces dormancy.

h) Rate of development may also be important in survival at low humidities, because, if the developmental rate is sufficiently high, the organism may be able to complete its development before it becomes excessively dehydrated.

i) When given a choice, many animals select an environment favorable for survival. Partially dehydrated insects tend to select lower temperatures than normal ones. Hence they are able to survive longer because the low temperature tends to decrease the loss of body water. Many organisms possess humidity receptors and are able to respond to a humidity gradient. In general, the response tends to bring the animal into an environment best suited for its metabolic processes.

10. Atmospheric humidity exerts a controlling influence on the distribution of animals. Those forms which possess no means of conserving water and are not protected from water loss by the presence of an impermeable covering must live in an environment with a high water content. Experimental evidence indicates that there are physiological differences in ecologically different species of animals. Xerophilic forms are able to survive longer exposures to low humidities than hygrophilic forms. Physiological differences in species appear to be correlated with the type of habitat which the animal can utilize.

LITERATURE CITED

ADOLPH, E. F. 1920. Egg-laying reactions in the pomace fly, *Drosophila*. Jour. Exper. Zöol., 31: 327–41.

———. 1932. The vapor tension relations of frogs. Biol. Bull., 62:112–25.

———. 1933. Exchanges of water in the frog. Biol. Rev., 8:224–40.

———. 1943. Physiological regulations. Lancaster, Pa.: Jacques Cattell Press.

AHMAD, T. 1936. The influence of ecological factors on the Mediterranean flour moth, *Ephestia kuehniella* and its parasite, *Nemenitus canescens*. Jour. Animal Ecol., 5:67–93.

ANDERSEN, K. TH. 1930. Der Einfluss der Temperatur und der Luftfeuchtigkeit auf die Dauer der Eizeit. I. Beitrag zu einer exakten Biologie des linierten Graurüsslers (*Sitona lineata* L.). Zeitschr. f. Morph. u. Ökol. Tiere, 17:649–76.

ANDERSON, D. B. 1936. Relative humidity or vapor pressure deficit. Ecology, 17:277–82.

BACHMETJEW, P. 1901. Experimentelle entomologische Studien vom physikalisch-chemischen Standpunkte aus, Band I: Temperaturverhältnisse bei Insekten. Leipzig: W. Engelmann.

BACOT, A., and LINZELL, L. 1919. The incubation period of the eggs of *Haematopinus asini*. Parasitology, 11:388–92.

BACOT, A., and MARTIN, C. J. 1924. The respective influences of temperature and moisture upon the survival of the rat flea, *Xenopsylla cheopis*, away from its host. Jour. Hyg., 23:98–105.

BEATTIE, M. V. F. 1928. Observations on the thermal death points of the blow fly at different relative humidities. Bull. Entom. Res., 18:397–403.

BEKIR, M. 1935. Experimentelle Untersuchungen über den Einfluss von Temperatur und Luftfeuchtigkeit auf die Sterblichkeit und Entwicklung des Rigelspinner, *Malacosoma neustria* L. Zeitschr. f. angew. Entom., 21:501–22.

BENTLEY, E. W. 1944. The biology and behavior of *Ptinus tectus* Boie (Coleoptera, Ptinidae) a pest of stored products. Jour. Exper. Biol., 20:152–58.

BERGER, B. 1907. Über die Widerstandsfähigkeit

der Tenebriolarven gegen Austrockung. Arch. f. d. ges. Physiol., 118:607–12.

BIRCH, L. C., and ANDREWARTHA, H. G. 1942. The influence of moisture on the eggs of *Austroicetes cruciata* with reference to its ability to survive desiccation. Australian Jour. Exper. Biol. and Med. Sci., 20:1–.

BODENHEIMER, F. S. 1929. Studien zur Epidemiologie, Ökologie und Physiologie der afrikanischen Wanderheuschrecke (*Schistocerca gregaria* Forsk.). Zeitschr. f. angew. Entom., 15:435–557.

———. 1931. Über die Temperaturabhängigkeiten von Insekten. III. Die Beziehungen der Vorzugstemperatur zur Luftfeuchtigkeit der Umgebung. Zeitschr. f. vergl. Physiol., 13:740–47.

BODINE, J. H. 1921. Factors influencing the water content and the rate of metabolism of certain Orthoptera. Jour. Exper. Zoöl., 32:137–64.

———. 1923. Hibernation in Orthoptera. I. Physiological changes during hibernation in certain Orthoptera. *Ibid.*, 37:457–76.

BREITENBRECHER, J. K. 1918. The relation of water to the behavior of the potato beetle in the desert. Pub. Carnegie Inst. Washington, No. 263, pp. 341–84.

BUXTON, P. A. 1924. Heat, moisture and animal life in deserts. Proc. Roy. Soc., London, B, 96:123–31.

———. 1930. Evaporation from the meal-worm (*Tenebrio:* Coleoptera) and atmospheric humidity. *Ibid.*, 106:560–77.

———. 1931*a*. The law governing the loss of water from an insect. Proc. Entom. Soc., London, 6: 27–31.

———. 1931*b*. The thermal death point of *Rhodnius* (Rhynchota, Heteroptera) under controlled conditions of humidity. Jour. Exper. Biol., 8:275–78.

———. 1932*a*. Terrestrial insects and the humidity of the environment. Biol. Rev., 7:275–320.

———. 1932*b*. The relation of adult *Rhodnius prolixus* (Reduviidae, Rhynchota) to atmospheric humidity. Parasitology, 24:429–39.

———. 1933. The effect of climatic conditions upon populations of insects. Trans. Roy. Soc. Trop. Med. and Hyg., 26:325–64.

BUXTON, P. A., and LEWIS, D. J. 1935. Climate and tsetse flies: laboratory studies upon *Glossina submorsitans* and *tachinoides*. Phil. Trans. Roy. Soc., London, B, 224:175–240.

CLARK, N. 1935. The effect of temperature and humidity upon the eggs of the bug, *Rhodnius prolixus* (Heteroptera; Reduviidae). Jour. Animal Ecol., 4:82–87.

DAVIDSON, J. 1932. Factors affecting oviposition of *Sminthurus viridis* L. (Collembola). Australian Jour. Exper. Biol. and Med. Sci., 10:1–16.

DAVIES, W. M. 1928. The effects of variation in relative humidity on certain species of Collembola. Jour. Exper. Biol., 6:79–86.

DAVIES, W. M., and HOBSON, R. P. 1935. Sheep blow fly investigations. I. The relationship of humidity to blow fly attack. Ann Applied Biol., 22: 279–93.

DECKER, G. C., and ANDRE, F. 1936. Studies on temperature and moisture as factors influencing winter mortality in adult chinch bugs. Iowa State College, Jour. Sci., 10:403–20.

DICK, J. 1937. Oviposition in certain Coleoptera. Ann. Applied Biol., 24:762–96.

DURIG, A. 1901. Wassergehalt und Organfunction. Arch. f. d. ges. Physiol., 85:401–504.

EASTHAM, L. E. S., and MCCULLY, S. B. 1943. The oviposition responses of *Calandra granaria* Linn. Jour. Exper. Biol., 20:35–42.

ELWYN, A. 1917. Effect of humidity on pupal duration and on pupal mortality of *Drosophila ampelophila* Loew. Bull. Amer. Mus. Nat. Hist., 37:347–53.

EVANS, A. C. 1934. Studies on the influence of the environment on the sheep blow-fly, *Lucilia sericata* Meig. I. The influence of humidity and temperature on the egg. Parasitology, 26:365–77.

———. 1935. Studies on the influence of the environment on the sheep blow-fly, *Lucilia sericata* Meig. II. The influence of temperature and humidity on prepupae and pupae. *Ibid.*, 27:291–98.

———. 1936. Studies on the influence of the environment on the sheep blow-fly, *Lucilia sericata* Meig. IV. The indirect effect of temperature and humidity acting through certain competing species of blow-flies. *Ibid.*, 28:431–39.

EWER, D. W., and EWER, R. F. 1942. The biology and behavior of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. III. The effect of temperature and humidity on oviposition, feeding and duration of life cycle. Jour. Exper. Biol., 18:290–305.

FINK, D. E. 1925. Physiological studies on hibernation in the potato beetle, *Leptinotarsa decemlineata* Say. Biol. Bull., 49:381–406.

FRIEDEL, H. 1928. Ökologische und physiologische Untersuchungen an *Scutigerella immaculata* (Newp.). Zeitschr. f. Morph. u. Ökol. Tiere, 10: 738–97.

GEISTHARDT, G. 1937. Ueber die ökologische Valenz zweier Wanzenarten mit verschiedenem Verbreitungsgebeit. Zeitschr. f. Parasitenk., 9:151–202.

GÖSSWALD, K. 1938. Über den Einfluss von verschiedener Temperatur und Luftfeuchtigkeit auf die Lebensäusserungen der Ameisen. I. Die Lebensdauer ökologisch verschiedener Ameisenarten unter dem Einfluss bestimmter Luftfeuchtigkeit und Temperatur. Zeitschr. f. wiss. Zool. 151:337–81.

GORTNER, R. A. 1938. Outlines of biochemistry. 2d ed. New York: John Wiley & Sons.

GRAY, J. 1928. The role of water in the evolution of terrestrial vertebrates. Brit. Jour. Exper. Biol., 6:26–31.

GRISWOLD, G. H. 1944. Studies on the biology of the webbing clothes moth (*Tineola bisselliella* Hum.). Cornell Univ. Agric. Exper. Sta. Mem., No. 262, pp. 1–59.

GRISWOLD, G. H., and CROWELL, M. F. 1936. The effect of humidity on the development of the

webbing clothes moth (*Tineola bisselliella* Hum.). Ecology, 17:241–50.

GUNN, D. L. 1931. Temperature and humidity relations of the cockroach. Nature (London), 128: 186–87.

———. 1933. The temperature and humidity relations of the cockroach (*Blatta orientalis*). I. Desiccation. Jour. Exper. Biol., 10:274–85.

———. 1934. The temperature and humidity relations of the cockroach (*Blatta orientalis*). II. Temperature preference. Zeitschr. f. vergl. Physiol., 20:617–25.

———. 1935. The temperature and humidity relations of the cockroach. III. A comparison of temperature preference, and rates of desiccation and respiration of *Periplaneta americana*, *Blatta orientalis*, and *Blatella germanica*. Jour. Exper. Biol., 12:185–90.

———. 1937. The humidity reactions of the woodlouse, *Porcellio scaber* (Latreille). *Ibid.*, 14:178–86.

GUNN, D. L., and COSWAY, C. A. 1938. The temperature and humidity relations of the cockroach. V. Humidity preference. Jour. Exper. Biol., 15: 555–63.

GUNN, D. L., and KENNEDY, J. S. 1936. Apparatus for investigating the reactions of land arthropods to humidity. Jour. Exper. Biol., 13:450–59.

GUNN, D. L., and NOTLEY, F. B. 1936. The temperature and humidity relations of the cockroach. IV. Thermal death-point. Jour. Exper. Biol., 13: 28–34.

HALL, F. G. 1922. The vital limit of exsiccation of certain animals. Biol. Bull., 42:31–51.

HALL, F. G., and ROOT, R. W. 1930. The influence of humidity on the body temperature of certain poikelotherms. Biol. Bull., 58:52–58.

HAMILTON, A. G. 1936. The relation of humidity and temperature to the development of three species of African locusts—*Locusta migratoria migratorioides* (R. and F.), *Schistocerca gregaria* (Forsk.), *Nomadacris septemfasciata* (Serv.). Trans. Roy. Entom. Soc., London, 85:1–60.

HEADLEE, T. J. 1917. Some facts relative to the influence of atmospheric humidity on insect metabolism. Jour. Econ. Entom., 10:31–38.

HEFLEY, H. M. 1928. Differential effects of constant humidities on *Protoparce quinquemaculatus* Haworth, and its parasite *Winthemia quadripustulata* Fabricus. Jour. Econ. Entom., 21:213–21.

HODSON, A. C. 1937. Some aspects of the role of water in insect hibernation. Ecol. Mono., 7:271–315.

HOLDAWAY, F. G. 1932. An experimental study of the growth of populations of the "flour beetle," *Tribolium confusum* Duval, as affected by atmospheric humidity. Ecol. Mono., 2:261–304.

HOWES, N. H., and WELLS, G. P. 1934*a*. The water relations of snails and slugs. I. Weight rhythms in *Helix pomatia* L. Jour. Exper. Biol., 11:327–43.

———. 1934*b*. The water relations of snails and slugs. II. Weight rhythms in *Arion ater* L. and *Limax flavus* L. *Ibid.*, pp. 344–51.

HUBER, L. L.; NEISWANDER, C. R.; and SALTER, R. M. 1928. The European corn borer and its environment. Ohio Agric. Exper. Sta. Bull., No. 429. Pp. 196.

JANISCH, E. 1930. Experimentelle Untersuchungen über die Wirkung der Umweltfaktoren auf Insekten. I. Die Massenvermehrung der Baumwolleule *Prodenia littoralis* im Aegypten. Zeitschr. f. Morph. u. Ökol. Tiere, 17:339–416.

JOHNSON, C. G. 1940*a*. The longevity of the fasting bed-bug (*C. lectularius* L.) under experimental conditions and particularly in relation to the saturation deficiency law of water-loss. Parasitology, 32:239–70.

———. 1940*b*. Development, hatching, and mortality of the eggs of *Cimex lectularius* (Hemipt.) in relation to climate, with observations on the effects of preconditioning to temperature. *Ibid.*, pp. 127–73.

KENNEDY, J. S. 1937. The humidity reactions of the African migratory locust, *Locusta migratoria migratorioides* R. and F., gregarious phase. Jour. Exper. Biol., 14:187–97.

KOFOID, C. A. 1934. Climatic factors affecting the local occurrence of termites and their geographical distribution. Chap. ii, Termites and termite control. Berkeley: University of California Press.

KOIDSUMI, K. 1934. Experimentelle Studien über die Transpiration und den Wärmhaushalt bei Insekten. Mem. Fac. Sci. Agric. Taihoku Imp. Univ., 12:1–380.

KOJIMA, T. 1933. Studien zur Ökologie des Kieferspinners, *Dendrolinus pini* L. (Der Einfluss von Temperatur und Luftfeuchtigkeit auf Sterblichkeit und Entwicklungsdauer.) Zeitschr. f. angew. Entom., 20:329–53.

KOZHANTCHIKOV, I. 1936. On the periodicity of mass propagation of insect pests. The influence of temperature and humidity on the pupal development of *Agrotis segetum* Schiff. and on the quantity of the next generation. Bull. Plant Protection, Lenin Acad. Agric. Sci., Ser. 1, No. 19, pp. 5–36.

LEES, A. D. 1943. On the behavior of wireworms of the genus *Agriotes* Esch. (Coleoptera, Elateridae). I. Reactions to humidity. Jour. Exper. Biol., 20:43–53.

LEIGHLY, J. 1937. A note on evaporation. Ecology, 18:180–98.

LUDWIG, D. 1931. Studies on the metamorphosis of the Japanese beetle (*Popillia japonica* Newman). I. Weight and metabolism changes. Jour. Exper. Zoöl., 60:309–23.

———. 1936. The effect of desiccation on survival and metamorphosis of the Japanese beetle (*Popillia japonica* Newman). Physiol. Zoöl., 9: 7–42.

———. 1937. The effect of different relative humidities on the respiratory metabolism of the grasshopper *Chortophaga viridifasciata* De Geer. *Ibid.*, 10:342–51.

———. 1942. The effect of different relative humidities, during the pupal stage, on the reproductive capacity of the Luna moth, *Tropaea luna* L. *Ibid.*, 15:48–60.

LUDWIG, D. 1943. The effect of different relative humidities, during the pupal stage, on the reproductive capacity of the Cynthia moth, *Samia walkeri* Felder and Felder. *Ibid.*, **16**:381–88.

LUDWIG, D., and ANDERSON, J. M. 1942. Effects of different humidities, at various temperatures, on the early development of four saturniid moths (*Platysamia cecropia* Linnaeus, *Telea polyphemus* Cramer, *Samia walkeri* Felder and Felder, and *Callosamia promethea* Drury), and on the weights and water contents of their larvae. Ecology, **23**: 259–74.

LUDWIG, D., and LANDSMAN, H. M. 1937. The effect of different relative humidities on survival and metamorphosis of the Japanese beetle (*Popillia japonica* Newman). Physiol. Zoöl., **10**:171–79.

MACGILL, E. I. 1931. The biology of Thysanoptera with reference to the cotton plant. VII. The relation between temperature and humidity and the life cycle. Ann. Applied Biol., **18**:574–83.

MACLAGAN, D. S. 1932. An ecological study of the "lucerne flea" (*Smynthurus viridis* Linn.). Bull. Entom. Res., **23**:101–45.

MACLEOD, J. 1934. *Ixodes ricinus* in relation to its physical environment: the influence of climate on development. Parasitology, **26**:282–305.

———. 1935. *Ixodes ricinus* in relation to its physical environment. II. The factors governing survival and activity. *Ibid.*, **27**:123–44.

———. 1936. *Ixodes ricinus* in relation to its physical environment. IV. An analysis of the ecological complexes controlling distribution and activities. *Ibid.*, **28**:295–319.

MAERCKS, H. 1933. Der Einfluss von Temperatur und Luftfeuchtigkeit auf die Embryonalentwicklung der Mehlmottenschlupfwespe *Habrobracon juglandis* Ashmead. Arb. biol. Reichsanst. Landforstwirtschaft, **20**:347–90.

MANTON, S. M., and RAMSAY, J. A. 19 7. Studies on the Onychophora. III. The control of water loss in *Peripatopsis*. Jour. Exper. Biol., **14**:470–72.

MEHTA, D. R. 1930. Observations on the influence of temperature and humidity on the bionomics of *Dysdercus cingulatus* Fabr. Bull. Entom. Res., **21**:547–62.

MELLANBY, K. 1932*a*. The effect of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor* L., Coleoptera). Proc. Roy. Soc., London, B, **111**:376–90.

———. 193 *b*. Effects of temperature and humidity on the metabolism of the fasting bed-bug (*Cimex lectularius*), Hemiptera. Parasitology, **24**:419–28.

———. 1932*c*. The influence of atmospheric humidity on the thermal death-point of a number of insects. Jour. Exper. Biol., **9**:222–31.

———. 1933. The influence of temperature and humidity on the pupation of *Xenopsylla cheopis*. Bull. Entom. Res., **24**:197–202.

———. 1934. The site of water loss from insects. Proc. Roy. Soc., London, B, **116**:139–49.

———. 1935. The evaporation of water from insects. Biol. Rev., **10**:317–33.

———. 1941. The body temperature of the frog. Jour. Exper. Biol., **18**:55–61.

MENUSAN, H. 1934. Effects of temperature and humidity on the life processes of the bean weevil, *Bruchus obtectus* Say. Ann. Entom. Soc. Amer., **27**:515–26.

———. 1935. Effects of constant light, temperature, and humidity on the rate and total amount of oviposition of the bean weevil, *Bruchus obtectus* Say. Jour. Econ. Entom., **28**:448–53.

MILLER, D. F. 1930. The effect of temperature, relative humidity and exposure to sunlight upon the Mexican bean beetle. Jour. Econ. Entom., **23**: 945–55.

MILLER, M. A. 1938. Comparative ecological studies of terrestrial isopod Crustacea of the San Francisco Bay Region. Univ. Calif. Pub. Zoöl., **43**: 113–42.

NECHELES, H. 1924. Über Wärmeregulation bei wechselwarmen Tieren. Ein Beitrag zur vergleichenden Physiologie der Wärmeregulation. Arch. f. d. ges. Physiol., **204**:72–86.

PARKER, J. R. 1930. Some effects of temperature and moisture upon *Melanoplus mexicanus mexicanus* Saussure and *Camnula pellucida* Scudder (Orthoptera). Univ. Mont. Agric. Exper. Sta. Bull., No. 223, pp. 5–132.

PAYNE, N. M. 1926. Freezing and survival of insects at low temperatures. Quart. Rev. Biol., **1**:270–86.

———. 1927. Freezing and survival of insects at low temperatures. Jour. Morph., **43**:521–46.

———. 1928. Cold hardiness in the Japanese beetle, *Popillia japonica* Newman. Biol. Bull., **55**:163–79.

———. 1929. Absolute humidity as a factor in insect cold hardiness with a note on the effect of nutrition on cold hardiness. Ann. Entom. Soc. Amer., **22**:601–20.

PIELOU, D. P., and GUNN, D. L. 1940. The humidity behavior of the mealworm beetle, *Tenebrio molitor* L. I. The reaction to differences of humidity. Jour. Exper. Biol., **17**:286–94.

POWELL, T. E. 1931. An ecological study of the tobacco beetle, *Lasioderma serricorne* Fabr., with special reference to its life history and control. Ecol. Mono., **1**:333–93.

PYENSON, L., and SWEETMAN, H. L. 1931. The effects of temperature and moisture on the eggs of *Epilachna corrupta* Mulsant (Coccinellidae, Coleoptera). Bull. Brooklyn Entom. Soc., **26**:221–26.

RAMSAY, J. A. 1935*a*. Methods of measuring the evaporation of water from animals. Jour. Exper. Biol., **12**:355–72.

———. 1935*b*. The evaporation of water from the cockroach. *Ibid.*, pp. 373–83.

ROBINSON, W. 1926. Low temperature and moisture as factors in the ecology of the granary weevil, *Sitophilus granarius* L. and the rice weevil, *Sitophilus oryza* L. Univ. Minn. Agric. Exper. Sta. Tech. Bull., No. 41. Pp. 43.

———. 1927. Water binding capacity of colloids a definite factor in winter hardiness of insects. Jour. Econ. Entom., **20**:80–88.

———. 1928. Water conservation in insects. *Ibid.*, **21**:897–902.

ROUBAUD, E. 1909. Recherches biologiques sur les conditions de viviparité et de vie larvaire de *Glossina palpalis*, R. Desv. Compt. rend. Acad. sci. (Paris), **148**:195–97.

RUBTZOV, I. A. 1934. Fertility and climatic adaptations in Siberian grasshoppers. Bull. Entom. Res., **25**:339–48.

SACHAROV, N. L. 1930. Studies in cold resistance of insects. Ecology, **11**:505–17.

SALT, R. W. 1936. Studies on the freezing process of insects. Univ. Minn. Exper. Sta. Tech. Bull., No. 116. Pp. 41.

SAVELY, H. E. 1939. Ecological relations of certain animals in dead pine and oak logs. Ecol. Mono., **9**:323–85.

SCHOOF, H. F. 1941. The effects of various relative humidities on the life processes of the southern cowpea weevil, *Callosobruchus maculatus* (Fabr.) at 30° C., ±0.8°. Ecology, **22**:297–305.

SHELFORD, V. E. 1913. The reactions of certain animals to gradients of evaporating power of air. A study in experimental ecology. Biol. Bull., **25**: 79–120.

———. 1914. The importance of the evaporation of moisture in economic studies of insects. Jour. Econ. Entom., **7**:229–33.

SMART, J. 1935. The effects of temperature and humidity on the cheese skipper, *Piophila casei* (L.). J ur. Exper. Biol., **12**:384–88.

SOLOMON, M. E. 1937. Experiments on the effects of temperature and humidity on the survival of *Halotydeus destructor* (Tucker), Acarina, fam. Penthaleidae. Australian Jour. Exper. Biol. and Med. Sci., **15**:1–16.

SPEICHER, B. R. 1931. The effects of desiccation upon the growth and development of the Mediterranean flour-moth. Proc. Pa. Acad. Sci., **5**: 79–82.

STEINER, P. 1937. Hausbockuntersuchungen. I. Über den Einfluss von Temperatur und Feuchtigkeit auf das Eistadium und Bemerkungen zur Biologie der Imago. Zeitschr. f. angew. Entom., **23**:531–46.

SUBKLEW, W. 1934. Physiologische-experimentelle Untersuchungen an einigen Elateriden (*Agriotes obscurus* L., *Agriotes lineatus* L., *Corymbites tessellatus* L., und *Limonius* spec.). Zeitschr. f. Morph. u. Ökol. Tiere, **28**:184–228.

SWEETMAN, H. L. 1938. Physical ecology of the firebrat, *Thermobia domestica* (Packard). Ecol. Mono., **8**:285–311.

SWEETMAN, H. L., and WEDEMEYER, J. 1933. Further studies of the physical ecology of the alfalfa weevil, *Hypera postica* (Gyllenhal). Ecology, **14**: 46–60.

TALBOT, M. 1934. Distribution of ant species in the Chicago region with reference to ecological factors and physical toleration. Ecology, **15**:416–39.

THEODOR, O. 1936. On the relation of *Phlebotomus papatasii* to the temperature and humidity of the environment. Bull. Entom. Res., **27**:653–71.

THOMPSON, V., and BODINE, J. H. 1936. Oxygen consumption and rates of dehydration of grasshopper eggs (Orthoptera). Physiol. Zoöl., **9**:455–70.

THORNTHWAITE, C. W. 1940. Atmospheric moisture in relation to ecological problems. Ecology, **21**: 17–28.

THORSON, T., and SVIHLA, A. 1943. Correlation on the habitats of amphibians with their ability to survive the loss of body water. Ecology, **24**:374–81.

TISCHLER, W. 1939. Zur Ökologie der wichtigsten in Deutschland an getreide Schädlichen Pentatomiden. II. Zeitschr. f. Morph. u. Ökol. Tiere, **35**: 251–87.

TOWNSEND, M. T. 1926. The breaking-up of hibernation in the codling moth larva. Ann. Entom. Soc. Amer., **19**:429–39.

UTIDA, S. 1941. Studies on experimental population of the azuki bean weevil, *Callosobruchus chinensis* (L.). II. The effect of population density on progeny populations under different conditions of atmospheric moisture. Mem. Coll. Agric. Kyoto Imp. Univ., **49**:1–20.

UVAROV, B. P. 1931. Insects and climate. Trans. Entom. Soc. London, **79**:1–247.

WALOFF, N. 1941. The mechanisms of humidity reactions of terrestrial isopods. Jour. Exper. Biol., **18**:115–35.

WARDLE, R. A. 1930. Significant variables in the blow-fly environment. Ann. Applied Biol., **17**: 554–74.

WIGGLESWORTH, V. B. 1931*a*. The physiology of excretion in the blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). Jour. Exper. Biol., **8**:411–51.

———. 1931*b*. On the function of the so-called "rectal glands" of insects. Quart. Jour. Micr. Sci., **75**:131–50.

———. 1931*c*. The extent of air in the tracheoles of some terrestrial insects. Proc. Roy. Soc., London, **109**:354–69.

———. 1939. The principles of insect physiology. New York: E. P. Dutton & Co.

WILLIAMS, O. L. 1934. Some factors limiting the distribution of termites. Chap. iv, Termites and termite control. Berkeley: University of California Press.

WODSEDALEK, J. E. 1912. Life history and habits of *Trogoderma tarsale* (Melsh.) a museum pest. Ann. Entom. Soc. Amer., **5**:367–82.

ZWALUWENBURG, R. H. VAN. 1937. Summary of laboratory studies of *Anomala*, 1933–1935. Hawaiian Planter's Record, **41**:25–32.

ZWÖLFER, W. 1931. Studien zur Oekologie und Epidemiologie der Insekten. I. Die Kieferneule, *Panolis flammea* Schiff. Zeitschr. f. angew. Entom., **17**:475–562.

STUDIES ON THE BIOCHEMISTRY OF TETRAHYMENA
III. STRAIN DIFFERENCES

GEORGE W. KIDDER AND VIRGINIA C. DEWEY[1]
Arnold Biological Laboratory, Brown University

MANY papers have appeared in the literature in this country during the last fifteen years reporting investigations of what Furgason (1940) has called the "*Glaucoma-Colpidium*" group of ciliates. Most of these investigations have been of a physiological nature, and a number of differences in results appear. Many of these differences are undoubtedly due to differences between the strains maintained in the various laboratories, although this is not always apparent from the designations of the experimental organisms. In other cases apparent similarities between different "species" or even "genera" are magnified because the older terminology separated closely related forms. The exhaustive work of Furgason (1940) should have gone a long way in clearing up this confusion, but unfortunately a few authors have preferred to cling to generic and specific terms which seem to us to be indefensible. From a morphological standpoint, which has always determined the classification of the protozoa, it becomes quite obvious that the ciliates of the "*Glaucoma-Colpidium*" group do not fall within the generic limits of either the genus *Glaucoma* (type species *G. scintillans*) or the genus *Colpidium* (type species *C. colpoda*). Moreover, the cultural requirements of members of these two genera (*G. scintillans* and *C. campylum*) have been shown to differ so widely from those of the easily cultivated *Tetrahymena* (Kidder, 1941*b*; Peterson, 1942) that the structural differences pointed out by Furgason (1940) become even more convincing. We have made a study of pure cultures of a number of strains of *Tetrahymena* and *Glaucoma* and one strain of *C. campylum* during the past few years, and we are convinced that there is no excuse for continuing to use the names "*Colpidium campylum*" or "*Glaucoma pyriformis*" for ciliates falling within the generic limits of the organism described in detail by Furgason (1940). Whatever the biochemical and antigenic differences between the strains of *Tetrahymena* are shown to be, these ciliates should not be crowded into the two established genera mentioned.

In order to determine to our satisfaction the relationships between different strains of *Tetrahymena*, we have studied from a biochemical point of view six strains of *T. geleii* and two strains of *T. vorax*. We are at present investigating antigenic relationships in collaboration with Dr. C. A. Stuart of this laboratory and will report these findings in detail at a later time. The largest phase of the present investigation (amino acids in relation to growth) was made possible only by the recent discovery that a chemically defined nitrogen source would support growth in these ciliates (Kidder and Dewey, 1945*a*). It is therefore the purpose of this report to present our findings regarding the biochemical similarities and differences between the strains of *Tetrahymena* which we have had the opportunity to investigate.

[1] Aided by grants from the Morgan Edwards Fellowship Fund and the Manufacturers' Research Fund for Bacteriology and Protozoölogy at Brown University.

MATERIAL AND METHODS

1. STRAINS

Of the fourteen or more strains of *T. geleii*-like ciliates isolated in pure culture in this country, seven are still in existence, as far as we have been able to determine. Of these seven we have been able to study six strains, five of them through the co-operation of other workers in the field. The ciliate called by R. P. Hall and his colleagues "*Glaucoma piriformis*" (see Hall and Shottenfeld, 1941) is not included in the present study. As far as we are aware, the remaining seven strains (*Saprophilus oviformis*, three strains, and *Trichoda pura*, isolated by Glaser and Coria [1935]; *Glaucoma ficaria*, isolated by Johnson [1936]; *Colpidium striatum*, isolated by Marbarger [1943]; and *C. striatum*, used by Kline [1943]) have not been maintained.

It appears advisable to us to assign specific designations to each of the strains investigated, in order to obviate in the future any confusion which might arise as to the identity of the experimental organisms employed. These designations will be given below, together with the origins of the strains.

T. geleii E.—Isolated in pure culture in New York City by Dr. Alfred M. Elliott in 1933. This strain was called "*Colpidium striatum*" (Elliott, 1933). Cultures of strain E were sent to us by Dr. Elliott in 1938 and again in 1944.

T. geleii H.—Isolated in pure culture by Dr. Alford Hetherington at Pacific Grove, California, in 1931 under the name "*Colpidium campylum*" (Hetherington, 1933). It was sent to us in 1938 by Dr. Robert H. Hall.

T. geleii T.—Isolated in pure culture by Dr. John O. Thomas at Pacific Grove, California, in 1931. This is the strain investigated intensively by Furgason (1940) to which he gave the name "*Tetrahymena geleii*." Cultures of this strain were sent to us by Dr. W. H. Johnson in 1944.

T. geleii T-P.—This is a subculture of the Thomas strain (strain T) obtained from the Stanford Laboratories by Dr. Austin Phelps in 1934. Dr. Phelps sent us a culture of this strain in 1938, and we maintained it until 1943. In 1942 we sent a culture to Dr. Harold Kirby, and it was from him that we received the present cultures, sent in 1944. During the separation of the daughter-strain (T-P) from the parent-strain (T) there have occurred a number of biochemical changes between the two.

T. geleii W.—Isolated from Mill Pond, Woods Hole, Massachusetts, in 1940. It was sterilized and established in pure culture in this laboratory (Claff, 1940).

T. geleii GHH.—Sent to us for identification from Memphis, Tennessee, by Dr. M. A. Barber[2] in 1942. We identified it on morphological grounds as *Tetrahymena*, and on the basis of the experiments reported here it will be designated "*T. geleii* GHH."

T. vorax V.—Isolated and established in pure culture in this laboratory by Dr. D. M. Lilly in 1940. The original strain of this species (strain D), isolated in 1939 and first reported under the name of "*Glaucoma vorax* sp. nov." (Kidder, Lilly, and Claff, 1940), was lost in 1943. Strain V was obtained from the same source as strain D (a small pond in Cumberland, Rhode Island).

T. vorax PP.—Sent to us for identification from Memphis, Tennessee, by Dr. M. A. Barber in 1942. It had been maintained by Dr. Barber in association with a spirillum. It was sterilized and established in pure culture in this laboratory.

[2] These ciliates were used by Barber (1944) in studies on the nutrition of anopheline mosquitoes. No reference was made to the particular strain used.

Its similarity to strain V will be apparent from the results reported below.

Table 1 lists the various reports which have appeared in the literature on the eight strains of *Tetrahymena* used in this study, together with the names employed by the various authors in each case.

2. PROCEDURES

All experimental cultures were bacteria free and were incubated at 25.5° C. in Pyrex tubes (125 × 7 mm.). Because of the nature of this work it was found necessary to treat all glassware with dichromate-sulphuric cleaning solution, followed by thorough washing. All media were prepared with water twice distilled over permanganate in an all-Pyrex still. Inoculations were made with a bacteriological loop, delivering approximately 0.008 ml. of fluid.

Carbohydrates.—Most of the carbohydrates used were obtained from the Digestive Ferments Company. The exceptions were arabinose, rhamnose, glycogen, inulin, dextrin (Pfanstiehl, Special Chemicals Co.), ribose, lyxose, and fucose (Eastman Kodak Co.). All carbohydrates were used in 0.5 per cent concentrations in 1.0 per cent proteose-peptone broth to which was added the indicator, brom thymol blue (0.002 per cent). All of the pentoses and rhamnose, trehalose, and sorbitol were sterilized by filtration of solutions of 1.0 per cent concentration and added aseptically to equal volumes of previously autoclaved 2.0 per cent proteose-peptone broth plus indicator. The remainder of the carbohydrate media was sterilized by autoclaving at 15 pounds pressure for 12 minutes. The carbohydrate media were used in 4-ml. volumes and read after incubation for 5, 7, and 14 days. Results were recorded as plus (+) when the medium became acid (yellow), zero (0) when no change had occurred (green), and minus (−) when the medium became alkaline (blue).

Amino acids.—The sources of the nineteen amino acids used have been given in a previous report (Kidder and Dewey, 1945*a*). Each amino acid was dissolved in distilled water in a concentration such that the additions of equal amounts would result in the desired concentration in the mixture. To the amino acid mixtures were added eleven known growth factors, dextrose, salts, and a protein-free preparation of cerophyl extract containing Factors I and II (Dewey, 1944) and Factor III (Kidder and Dewey, 1945*b*). The preparation of cerophyl extract was a modification of that used earlier (Kidder and Dewey, 1945*a*), for it was found that the naturally occurring proteins could be eliminated without resort to the adsorption-elution procedure. Cerophyl (10 gm. per 100 ml. of water) was adjusted to pH 2.0–3.0 with sulphuric acid, autoclaved for 15 minutes, and filtered. When the filtrate was neutralized, a precipitate formed, and this was filtered off and discarded. The filtrate was again autoclaved for 15 minutes, adjusted to pH 2.0–3.0 with sulphuric acid, and the precipitate which formed was filtered off. This final filtrate was neutralized and was then ready for use. The ninhydrene test showed this preparation to be protein free, the protein having been removed, probably as the metaprotein. This preparation was added in the concentration of 1 part extract to 10 parts of final medium.

Table 2 gives the composition of the medium used to test for the indispensability of each acid. Variations in concentrations of certain of the amino acids will be noted where they occur.

All amino acid media were used at pH 6.8–6.9 in 2.0-ml. volumes, and populations were determined after incubation periods of 72 hours (except in the case of

T. geleii E, where 120-hour periods were used) by the direct-counting technique described earlier (Kidder, 1941*a*). The small volumes in tubes were used in preference to the growth flasks previously employed (Kidder, 1941*a*; Kidder and Dewey, 1944) because of the expense and difficulty of obtaining large quantities of the amino acids. It was also felt that the rubber of the vaccine tip used in the flasks might possibly contribute minute amounts of the substances under investi-

TABLE 1

PUBLISHED NOMENCLATURE OF *Tetrahymena*

STRAIN	REFERENCE	NAME EMPLOYED
	Tetrahymena geleii	
H.....	Hetherington (1933); Elliott (1935*a*, 1935*b*); Hall and Elliott (1935); R. P. Hall (1937, 1939, 1940, 1942); Loefer and Hall (1936); Bond (1933); Loefer (1938); R. H. Hall (1938); Kidder (1939*a*); Kidder and Stuart (1939); Kidder *et al.* (1940); Hall and Loefer (1940); Tanzer (1941)	*Colpidium campylum*
	Hetherington (1934, 1936); Johnson (1936); Jahn (1936)	*Glaucoma pyriformis* or *G. piriformis*
	Kidder (1941*a*); Claff *et al.* (1941); Lilly (1942); Ormsbee (1942)	*Tetrahymena geleii*
E.....	Elliott (1933, 1935*a*, 1935*c*, 1938, 1939); Kidder *et al.* (1940); Tanzer (1941)	*Colpidium striatum*
T.....	Taylor *et al.* (1933)	*Colpidium campylum*
	Furgason (1936); Giese (1938)	*Tetrahymena glaucomiformia*
	Brown (1940)	*Glaucoma pyriformis*
	Furgason (1940); Baker and Baumberger (1941); Van Neil *et al.* (1942); Johnson and Baker (1943); Thomas (1943)	*Tetrahymena geleii*
T-P.....	Phelps (1935, 1936); Kidder (1939*b*); Kidder *et al.* (1940)	*Glaucoma pyriformis*
	Lilly (1942)	*Tetrahymena geleii* (strain P)
W.....	Dewey (1941, 1944); Kidder (1941*b*); Kidder and Dewey (1942, 1944, 1945*a*, 1945*b*)	*Tetrahymena geleii*
	Claff (1940); Kidder *et al.* (1940)	*Glaucoma ficaria*
GHH....	Barber (1944) (see n. 2)	*Tetrahymena geleii*
	Tetrahymena vorax	
V.....	Kidder and Dewey (1942)	*Tetrahymena vorax*
PP.....	Barber (1944) (see n. 2)	*Tetrahymena geleii*

gation. All experiments reported represent the results of at least three serial transplants (to eliminate carry-over of substances), with the exception of those experiments in which added amino acids were being tested. In those cases transplants were made from a medium in which the amino acid being studied was absent.

Thiamine requirement.—To test the ability of the various strains to synthesize thiamine, the same procedure was followed as had been used previously with *T. geleii* W (Kidder and Dewey, 1944). Dethiaminized gelatin (Harris) plus tryptophane was used with dethiaminized alfalfa meal extract, with and without added thiamine, and at least three serial transplants made in each case.

TABLE 2

COMPOSITION OF COMPLETE AMINO ACID MEDIUM

Amino Acid	Mg. Per Cent	Known Growth Factor	γ Per Cent
dl-Alanine	43.5	Biotin methyl ester	0.005
l(+)-Arginine monohydrochloride	41.0	Calcium pantothenate	10.00
dl-Aspartic acid	17.0	Nicotinic acid	10.00
l(−)-Cystine	1.0	*i*-Inositol	100.00
dl-Glutamic acid	25.0	Choline chloride	100.00
Glycine	127.0	*p*-Amino benzoic acid	10.00
l(+)-Histidine monohydrochloride	5.0	Pyridoxine hydrochloride	10.00
l(−)-Hydroxyproline	10.0	Uracil	10.00
dl-Isoleucine	17.5	Folic acid concentrate	0.10
l(−)-Leucine	17.5	Riboflavin	10.00
dl-Lysine monohydrochloride	30.0	Thiamine hydrochloride	10.00
dl-Methionine	17.0	**Inorganic Salts**	**Mg. Per Cent**
dl-Phenylalanine	7.0	$MgSO_4 \cdot 7H_2O$	10.00
l(−)-Proline	47.5	K_2HPO_4	10.00
dl-Serine	2.0	$CaCl_2 \cdot 2H_2O$	5.00
dl-Threonine	10.0	$FeCl_3 \cdot 6H_2O$	0.125
l(−)-Tryptophane	5.0	$MnCl_2 \cdot 4H_2O$	0.005
l(−)-Tyrosine	10.0	$ZnCl_2$	0.005
dl-Valine	10.0		

To the above was added 200 mg. per cent dextrose and 1 part of the protein-free cerophyl extract in 10 parts of final medium

All data, except those showing the effects of single omissions of amino acids from the complete (nineteen) list, are summarized in the following manner. Minus (−) represents no growth or very low growth, not more than 1,000 cells per milliliter; one plus sign (+) represents growth up to 10,000 cells per milliliter; two plus signs (++) growth up to 25,000 cells per milliliter; three plus signs (+++) growth up to 125,000 cells per milliliter; four plus signs (++++) growth up to 500,000 cells per milliliter.

RESULTS

CARBOHYDRATE FERMENTATION

The elaboration of specific carbohydrases represents distinct synthetic capacity, and differences in this capacity form important diagnostic tests in the field of bacteriology. Twenty-seven carbohydrates, representing eight groups, were tested to determine the ability of the eight strains of *Tetrahymena* to cause

their fermentation, with the aim of using these results for diagnostic purposes.

From Table 3 it will be seen that none of the ciliates studied fermented carbohydrates in Groups I (pentoses), II (desoxyhexoses), V (trisaccharides), VII (polyhydric alcohols), or VIII (glucoside). All of the hexoses (Group III) were fermented; levulose (slowly by *T. vorax* V and PP), dextrose and mannose by all eight strains, while galactose was fermented by *T. geleii* E, *T. vorax* V and PP, and very slowly by *T. geleii* H. Only two disaccharides (Group IV) were fermented, maltose by all strains, and lactose by the two strains of *T. vorax*. All the ciliates

TABLE 3*

CARBOHYDRATE FERMENTATION

Group	Carbohydrate	*T. geleii* H			*T. geleii* E			*T. geleii* T-P			*T. geleii* T			*T. geleii* W			*T. geleii* GHH			*T. vorax* V			*T. vorax* PP		
		Days																							
		5	7	14	5	7	14	5	7	14	5	7	14	5	7	14	5	7	14	5	7	14	5	7	14
I	Arabinose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Xylose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Ribose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Lyxose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
II	Rhamnose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Fucose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
III	Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Levulose	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	o	+	+
	Galactose	−	o	o	o	+	+	−	−	−	−	−	−	−	−	−	o	−	−	+	+	+	+	+	+
	Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	o	+	+
IV	Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	+	+	+	+	+
	Lactose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+
	Sucrose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Cellobiose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Melibiose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Trehalose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
V	Raffinose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Melezitose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
VI	Dextrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Glycogen	+	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Inulin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
VII	Dulcitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Sorbitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Mannitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Adonitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
VIII	Salicin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No added carbohydrate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* Plus sign (+) = acid production; zero (o) = no change in pH of medium; minus sign (−) = alkaline reaction.

fermented dextrin, glycogen, and starch (purified potato starch), but inulin was never fermented (Group VI, polysaccharides).

It becomes apparent from the results presented (Table 3) that few of the carbohydrates tested are of diagnostic importance. It is possible, however, to divide the eight strains of ciliates into four distinct groups by the use of galactose, lactose, and starch. *T. geleii* H is galactose slow positive, lactose negative, and starch positive. By the fourteenth day at 25.5° C. there has occurred a reversal from an acid to an alkaline reaction. None of the other strains show this reversal by the fourteenth day. *T. geleii* T, T-P, W, and GHH fall into one group (galactose negative, lactose negative, starch positive with no return to alkalinity by 14 days). *T. geleii* E is galactose positive, lactose negative, and starch positive. Both strains of *T. vorax* are alike in their ability to ferment lactose (galactose positive, lactose positive, starch positive).

EFFECT OF OMISSIONS OF SINGLE AMINO ACIDS

The usual method for determining whether or not a given amino acid is indispensable for a given organism is the omission of each amino acid singly from a complete diet. The standard definition of an essential amino acid is (Rose, 1938) one which cannot be synthesized by the animal organism out of the materials ordinarily available at a speed commensurate with the demands of normal growth. While both the method and the definition leave much to be desired (Hegsted, 1944), they are useful as a starting-point in the exploration of amino acid requirements. For all the strains of *Tetrahymena* reported here, a growth of approximately 200,000 organisms per milli-

TABLE 4

EFFECT OF OMISSION OF SINGLE AMINO ACIDS

Amino Acid Omitted	Population in Third Transplant at 72 Hours (Cells/Ml.)							
	T. geleii						*T. vorax*	
	H	E	T-P	T	W	GHH	V	PP
None	390,000	750	260	420,000	320,000	220,000	460,000	320,000
Alanine	410,000	320	9,550	390,000	270,000	250,000	410,000	395,000
Arginine	2,000	900	6,200	1,100	6,500	900	1,500	2,000
Aspartic acid	420,000	600	5,000	310,000	290,000	180,000	380,000	360,000
Cystine	300,000	0	0	260,000	240,000	160,000	385,000	310,000
Glutamic acid	380,000	1,100	115,000	350,000	200,000	190,000	360,000	390,000
Glycine	560	50	4,200	80	260	700	10,000	13,000
Histidine	0	100	0	0	0	350	0	0
Hydroxyproline	360,000	45	37,000	310,000	240,000	290,000	410,000	300,000
Isoleucine	80	260	0	0	0	55	0	0
Leucine	20	0	0	5	0	60	0	0
Lysine	0	20	0	0	0	0	0	0
Methionine	160	170	50	10	15	380	0	0
Phenylalanine	200	400	370	30	20	1,200	950	20
Proline	360,000	80	10,000	380,000	210,000	275,000	420,000	290,000
Serine	310,000	0	0	270,000	300,000	190,000	360,000	350,000
Threonine	20	280	0	0	0	350	0	0
Tryptophane	60	240	120	35	300	1,450	0	0
Tyrosine	270,000	2,200	305,000	380,000	210,000	250,000	265,000	305,000
Valine	30	400	350	0	0	200	0	0

liter may be considered as being normal growth. The low growth in the complete medium shown by *T. geleii* E and T-P makes it necessary to consider these cases separately.

It may be seen (Table 4) that for all the strains, with the two exceptions noted above, arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine may be considered to be essential amino acids. This would not be true, however, if an essential amino acid were defined as one essential for the life of the organism. That the low growth observed in the absence of certain of the essential amino acids may be an artifact due to contamination of some of the naturally occurring isomers with other amino acids is apparent from the data presented in Table 5. The *l*(−)-leucine used was found to be contaminated with methionine, phenylalanine, and tryptophane. To what extent this is true of others of the natural amino acids used remains to be determined before the absolute requirements of these ciliates can be stated. Care must also be taken with synthetic leucine, which may contain considerable quantities of isoleucine (Hegsted and Wardwell, 1944; Kidder and Dewey, 1945*a*).

TABLE 5

SUBSTITUTION OF *dl*-LEUCINE FOR *l*(−)-LEUCINE IN THE MIXTURES OF EIGHTEEN AMINO ACIDS TO DETERMINE THE ESSENTIALITY OF METHIONINE, PHENYLALANINE, AND TRYPTOPHANE

Amino Acid Omitted	Population in Third Transplant at 72 Hours (Cells/Ml.)					
	T. geleii				*T. vorax*	
	H	T	W	GHH	V	PP
Methionine	0	0	0	0	0	0
Phenylalanine	5	10	0	1,100	0	0
Tryptophane	15	20	40	850	0	0

ATTEMPTS TO SUBSTITUTE FOR INDISPENSABLE AMINO ACIDS

Because the omission of glycine resulted in extremely low growth in all cases and because cystine and serine appeared to be absolute requirements for two strains (Table 4), these amino acids were selected for study along with the ten amino acids indispensable for mammals (basic ten for *T. geleii* W [Kidder and Dewey, 1945*a*]). It was found that only *T. geleii* W and GHH were capable of maintenance in these ten amino acids (Tables 10 and 11). The other strains failed entirely (Tables 6, 7, 8, 9, 12, 13). The addition of serine (10 mg. per cent), on the other hand, permitted good growth of all strains. Glycine (10 mg. per cent) could replace serine for both strains of *T. vorax* but had little or no effect on the strains of *T. geleii*, with the exception of *T. geleii* GHH, in which there was a slight improvement of growth over that in the ten amino acids alone. It is probable, however, that large amounts of glycine may stimulate growth, as has been reported for *T. geleii* W (Kidder and Dewey, 1945*a*). This appears to be a nonspecific effect produced by the addition of a large amount of amino nitrogen, which is not true in the case of serine. For only *T. geleii* T-P could cystine replace

serine, although there was a slight improvement in the growth of *T. geleii* GHH when cystine was added to the basic ten amino acids.

permit the conclusion that serine could substitute for one of the essential amino acids. It will be noted, however, that *T. geleii* E and GHH gave evidence of abil-

TABLE 6

Tetrahymena geleii H: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−	−	−	−	−
Ten plus glycine	−	−	−	−	−	−	−	−	−	−	−
Ten plus serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and glycine	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and serine	+++	−	−	−	−	−	−	−	−	−	−
Ten plus glycine and serine	−	−	−	−	−	−	−	−	−	−	+
Ten plus cystine, glycine, and serine	++++	+	−	−	−	−	+	−	−	−	+++

* *l*(−)-Cystine = 2.0 mg. per cent; glycine = 10 mg. per cent; *dl*-serine = 10 mg. per cent; ten = *l*(+)-arginine monohydrochloride = 82 mg. per cent; *l*(+)-histidine monohydrochloride = 10 mg. per cent; *dl*-isoleucine = 35 mg. per cent; *l*(−)-leucine = 35 mg. per cent; *dl*-lysine monohydrochloride = 60 mg. per cent; *dl*-methionine = 34 mg. per cent; *dl*-phenylalanine = 14 mg. per cent; *dl*-threonine = 20 mg. per cent; *l*(−)-tryptophane = 10 mg. per cent; *dl*-valine = 20 mg. per cent.

TABLE 7

Tetrahymena geleii E: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−	−	−	−	−
Ten plus glycine	−	−	−	−	−	−	−	−	−	−	−
Ten plus serine	++++	++	−	+	+	−	−	+	+	++	++
Ten plus cystine and glycine	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus glycine and serine	+++	−	−	−	−	−	−	−	−	−	+
Ten plus cystine, glycine, and serine	+++	+	−	−	+	−	−	−	++	+	+++

* For quantities see note to Table 6.

Since the addition of serine to the ten essential amino acids gave such a marked improvement in growth, the effect of the omission of each of the ten singly from the mixture of eleven was tested. In no case was growth obtained which would

ity to synthesize certain of the essential amino acids at a slow rate, in the presence of serine. Since the serine used was synthetic, it is impossible that this effect was due to the introduction of essential amino acids as contaminants. This phase

of the work requires further investigation before a decision can be reached as to definite requirements. From Tables 12 and 13 it may be seen that the same ten amino acids must be considered essential for these strains.

three amino acids were somewhat more effective than any of them singly in the case of *T. geleii* T-P and GHH in permitting synthesis of essential amino acids. A notable effect is seen in the ability of all strains, except *T. vorax* V, to

TABLE 8

Tetrahymena geleii T-P: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	+++	−	−	−	−	−	−	+	−	+	−
Ten plus glycine	−	−	−	−	−	−	−	+	−	−	−
Ten plus serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and glycine	−	+	−	+	−	−	−	+	+	+	+
Ten plus cystine and serine	++++	++	−	−	++	−	−	+	++	+	+++
Ten plus glycine and serine	−	++	−	+	++	−	+	+	++	++	+++
Ten plus cystine, glycine, and serine	+++	−	−	−	−	−	−	−	−	−	++

* For quantities see note to Table 6.

TABLE 9

Tetrahymena geleii T: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−	−	−	−	−
Ten plus glycine	−	−	−	−	−	−	−	−	−	−	−
Ten plus serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and glycine	−	−	−	−	−	−	−	−	−	−	+
Ten plus cystine and serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus glycine and serine	−	−	−	−	−	−	−	−	−	−	+
Ten plus cystine, glycine, and serine	+++	−	−	−	−	−	−	−	−	−	++

* For quantities see note to Table 6.

Although similar effects might have been expected with all strains as with the two strains of *T. vorax* when glycine was used in the place of serine, it was only in the case of *T. geleii* GHH that glycine was at all active. Cystine was active only with *T. geleii* T-P. Combinations of these grow in the absence of valine when cystine, glycine, and serine are all present.

EFFECT OF ADDITION OF DISPENSABLE AMINO ACIDS

The effects described above for cystine, glycine, and serine could not be re-

TABLE 10

Tetrahymena geleii W: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	+	−	−	−	−	−	−	−	−	−	−
Ten plus glycine	−	−	−	−	−	−	−	−	−	−	−
Ten plus serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and glycine	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus glycine and serine	++	+	−	−	−	−	−	−	−	−	−
Ten plus cystine, glycine, and serine	+++	+	−	−	−	−	−	−	−	−	++

* For quantities see note to Table 6.

TABLE 11

Tetrahymena geleii GHH: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	+	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	++	++	−	−	−	−	−	−	−	+	−
Ten plus glycine	++	+	−	−	−	−	−	−	++	−	−
Ten plus serine	++++	++	−	−	+	−	−	+	++	+	++
Ten plus cystine and glycine	−	+	−	+	+	++	−	+	++	+	++
Ten plus cystine and serine	++++	+	−	−	++	−	++	++	+++	++	+++
Ten plus glycine and serine	+++	++	−	+	+	−	−	+	++	−	++
Ten plus cystine, glycine, and serine	+++	+	−	+	+	−	+	+	++	+	+++

* For quantities see note to Table 6.

TABLE 12

Tetrahymena vorax V: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−	−	−	−	−
Ten plus glycine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and glycine	++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and serine	−	−	−	−	−	−	−	−	−	−	−
Ten plus glycine and serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine, glycine, and serine	+++	−	−	−	−	−	−	−	−	−	−

* For quantities see note to Table 6.

produced by any of the dispensable amino acids (alanine, aspartic acid, glutamic acid, hydroxyproline, proline, or tyrosine) (see Tables 14–21). Indeed, when members of this group of amino acids are present as well as one or more noted that the stimulatory amino acids appear to act synergistically in the release of the inhibition caused by the dispensable amino acids. For example, both glycine and serine are stimulatory to *T. vorax* V (Table 20) while cystine is

TABLE 13

Tetrahymena vorax PP: EFFECT OF OMISSIONS OF INDISPENSABLE AMINO ACIDS

Medium*	Amino Acid Omitted										
	None	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine
Ten	−	−	−	−	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−	−	−	−	−
Ten plus glycine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus cystine and glycine	+	−	−	−	−	−	−	−	+	−	−
Ten plus cystine and serine	++++	−	−	−	−	−	−	−	−	−	−
Ten plus glycine and serine	++++	−	−	−	−	−	−	−	+	−	−
Ten plus cystine, glycine, and serine	++++	−	−	−	−	−	−	−	+	+	+

* For quantities see note to Table 6.

TABLE 14

Tetrahymena geleii H: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxyproline	Proline	Tyrosine
Ten	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−
Ten plus glycine	−	−	−	−	−	−	−
Ten plus serine	++++	−	+++	++	++	++++	−
Ten plus cystine and glycine	−	−	−	−	−	−	−
Ten plus cystine and serine	+++	++	++++	+++	+++	−	−
Ten plus glycine and serine	−	++++	++++	++++	++++	++++	+++
Ten plus cystine, glycine, and serine	++++	++++	++++	++++	++++	++++	++++

* For quantities see note to Table 6.

of the stimulatory amino acids, the former are found to be generally somewhat inhibitory. This is particularly true of *T. geleii* E (Table 15), in which case both cystine and glycine also appear to be inhibitory. The only good growth occurs where serine is present. It will also be slightly inhibitory. Any of the dispensable amino acids or cystine causes partial inhibition of growth in the presence of glycine or serine, but that inhibition is released when both glycine and serine are present. Not all the reactions are so clear cut as that; there seem to be interactions

TABLE 15

Tetrahymena geleii E: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxy-proline	Proline	Tyrosine
Ten	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−
Ten plus glycine	−	−	−	−	−	−	−
Ten plus serine	++++	−	++++	++	++	++++	−
Ten plus cystine and glycine	−	−	−	−	−	−	−
Ten plus cystine and serine	++++	−	−	−	−	−	−
Ten plus gylcine and serine	+++	+++	+++	+++	+++	+++	+++
Ten plus cystine, glycine, and serine	+++	++	++	++	+	+	+

* For quantities see note to Table 6.

TABLE 16

Tetrahymena geleii T-P: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxy-proline	Proline	Tyrosine
Ten	−	−	−	−	−	−	−
Ten plus cystine	+++	−	++	++	+	−	−
Ten plus glycine	−	−	−	−	−	−	−
Ten plus serine	++++	+	++++	++++	++++	++++	−
Ten plus cystine and glycine	−	−	−	+	−	−	+
Ten plus cystine and serine	++++	++++	++++	++++	++++	++++	++++
Ten plus glycine and serine	−	++	+++	+++	++++	+++	+++
Ten plus cystine, glycine, and serine	+++	+++	+++	+++	+++	+++	+++

* For quantities see note to Table 6.

TABLE 17

Tetrahymena geleii T: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxy-proline	Proline	Tyrosine
Ten	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	+	−	−	−
Ten plus glycine	−	−	−	−	−	−	−
Ten plus serine	++++	−	++	++	+	++	−
Ten plus cystine and glycine	−	−	+	+	−	−	−
Ten plus cystine and serine	++++	++	+++	+	++	++	++
Ten plus glycine and serine	−	+++	+++	+++	+++	+++	+++
Ten plus cystine, glycine, and serine	+++	+++	+++	+++	+++	+++	+++

* For quantities see note to Table 6.

TABLE 18

Tetrahymena geleii W: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxy-proline	Proline	Tyrosine
Ten	++	−	−	−	−	−	−
Ten plus cystine	+	−	−	+	−	−	−
Ten plus glycine	−	−	−	−	−	−	−
Ten plus serine	++++	++	+++	+++	+++	+++	++
Ten plus cystine and glycine	−	−	−	−	−	−	−
Ten plus cystine and serine	++++	+	++	+	++	++	++
Ten plus glycine and serine	++	+++	+++	+++	+++	+++	+++
Ten plus cystine, glycine, and serine	+++	+++	+++	+++	+++	+++	+++

* For quantities see note to Table 6.

TABLE 19

Tetrahymena geleii GHH: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxy-proline	Proline	Tyrosine
Ten	+	+	+	−	−	−	−
Ten plus cystine	++	+	+	+	+	+	−
Ten plus glycine	++	−	−	−	−	−	−
Ten plus serine	++++	+++	+++	++++	++++	+++	−
Ten plus cystine and glycine	−	−	−	−	−	−	−
Ten plus cystine and serine	++++	+++	+++	++++	+++	+++	+++
Ten plus glycine and serine	+++	+++	+++	+++	++++	+++	++++
Ten plus cystine, glycine, and serine	+++	+++	+++	+++	+++	+++	+++

* For quantities see note to Table 6.

TABLE 20

Tetrahymena vorax V: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxy-proline	Proline	Tyrosine
Ten	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−
Ten plus glycine	++++	+	+	+	+++	+	+++
Ten plus serine	++++	+++	+	++	+++	+++	++++
Ten plus cystine and glycine	++	+	+	+	+	−	−
Ten plus cystine and serine	−	+++	++	++	++	+++	++
Ten plus glycine and serine	++++	+++	+++	++++	++++	++++	++++
Ten plus cystine, glycine, and serine	+++	++++	++++	++++	++++	++++	++++

* For quantities see note to Table 6.

and antagonisms among the stimulatory amino acids which vary according to the dispensable amino acid present. This makes generalizations both difficult and dangerous. The data presented here represent a preliminary exploration of the possibilities and cannot serve as the basis for the drawing of final conclusions.

THIAMINE SYNTHESIS

Precise information regarding the necessity for any one of the chemically known vitamins for the growth of *Tetrahymena* is confined to thiamine. This is due to the fact that complete media devoid of other chemically defined growth factors cannot be used. Even with the use of amino acid mixtures as a base medium, the necessity for the inclusion of plant extracts makes it difficult or impossible to determine the requirements for most of the known vitamins. There still remains the difficulty of removing known factors.

Because of the lability of thiamine to heat at high pH it has been possible to prepare media devoid of thiamine but complete in every other way for *Tetrahymena*. Following the procedure used to determine the effects of thiamine on the growth of *T. geleii* W (Kidder and Dewey, 1944), all eight strains were tested (six for the first time) for their ability to grow in the absence of thiamine.

Indefinitely transplantable growth at a high level is obtained with all six strains of *T. geleii* and the two strains of *T. vorax* used in this investigation, in dethiaminized gelatin (Harris) plus tryptophane and dethiaminized alfalfa extract. The addition of thiamine made no appreciable difference in the early growth phases, although added thiamine prolonged the stationary phase and delayed the onset of culture decline (Johnson and Baker, 1943). It can be concluded, therefore, that *T. geleii* H, E, T, T-P, and GHH and *T. vorax* PP are similar to *T. geleii* W (Kidder and Dewey, 1942, 1944) and to *T. vorax* V (Kidder and Dewey, 1942) in their ability to synthesize thiamine. Thiamine requirement may be dismissed as a means for differentiating strains.

TABLE 21

Tetrahymena vorax PP: EFFECT OF AMINO ACIDS ON GROWTH

Medium*	Amino Acid Added						
	None	Alanine	Aspartic Acid	Glutamic Acid	Hydroxy-proline	Proline	Tyrosine
Ten	−	−	−	−	−	−	−
Ten plus cystine	−	−	−	−	−	−	−
Ten plus glycine	++++	−	+	−	+++	−	+
Ten plus serine	++++	++++	++	+++	++++	++++	++++
Ten plus cystine and glycine	+	+	+	+	+	−	−
Ten plus cystine and serine	++++	++++	+++	+++	+++	+++	++++
Ten plus glycine and serine	++++	++++	++++	+++	++++	++++	++++
Ten plus cystine, glycine, and serine	++++	++++	++++	++++	++++	++++	++++

* For quantities see note to Table 6.

DISCUSSION

The simplicity of tests for carbohydrate fermentation makes this a popular method of diagnosis. Such tests would be desirable for application to differentia-

tion of protozoa. However, the fact that the enzymes responsible for the fermentation of carbohydrates may be adaptive —i.e., an enzyme produced only when the specific substrate is present—must be taken into account when carbohydrate-fermentation tests are set up for diagnostic purposes. It is also possible that other factors may influence enzyme production. Such physical influences as time and temperature of incubation and pH of the medium or such chemical factors as the source and concentration of nitrogenous nutrients, concentration of carbohydrate, and the presence of growth-promoting substances may influence the acid production, which is the criterion for carbohydrate fermentation. The apparent acid formation may also be influenced by the buffering capacity of the medium and the indicator used. All these factors must be taken into consideration when results from various laboratories are compared. For any given type of organism some standard medium should be formulated when such data are to be used for diagnostic purposes.

Any of the above-mentioned factors may be responsible for the discrepancies in results regarding carbohydrate fermentation which are found in the literature on *Tetrahymena*. It is true, of course, that some of the differences are due to strain differences. Table 22 gives a comparison of results of other workers regarding carbohydrate fermentation. While there is general agreement as to the inability of *T. geleii* or *T. vorax* to ferment arabinose, xylose, rhamnose, sucrose, melezitose, mannitol, inulin, or salicin and their ability to ferment levulose and dextrose, there is disagreement regarding the other carbohydrates. It is obvious that the highly buffered medium used by Elliott (1935*c*) gives results different from those obtained with less buffer. This difference is in part due, no

TABLE 22

COMPARISON OF DATA FROM THE LITERATURE ON CARBOHYDRATE FERMENTATION BY *Tetrahymena*

Strain	Reference	Carbohydrate						
		Galactose	Mannose	Maltose	Lactose	Cellobiose	Dextrin	Starch
Glaucoma piriformis	Colas-Belcour and Lwoff (1925)	+	*	+	−	*	*	−
Glaucoma ficaria	Johnson (1935)	+ Slow	−	+	*	*	+	+
Glaucoma piriformis	Johnson (1935)	+ Slow	−	+	*	*	+	+
T. geleii H (buffered)	Elliott (1935*c*)	−	+	+	−	*	−	+
T. geleii H (less buffer)	Elliott (1935*c*)	*	*	+	−	*	+	*
T. geleii H	Present report	+ Slow	+	+	−	−	+	+
T. geleii E (buffered)	Elliott (1935*c*)	−	+	+	−	*	−	+
T. geleii E (less buffer)	Elliott (1935*c*)	*	*	+	−	*	+	*
T. geleii E	Present report	+	+	+	−	−	+	+
T. geleii W	Kidder (1941*b*)	−	*	+	−	+	*	+
T. geleii W	Present report	−	+	+	−	−	+	+
T. vorax D	Kidder (1941*b*)	−	*	+	−	+	*	+
T. vorax V	Present report	+	+	+	+	−	+	+

*Not tested.

doubt, to the obscuring of acid production by the buffering effect and also to decreased growth due to the high concentration of phosphate used. In setting up carbohydrate media for the purpose of differentiation of strains or species the aim is to accentuate the acid production.

Even with the omission of the data discussed above, there is disagreement regarding the fermentability of galactose, mannose, lactose, and cellobiose. In the case of galactose, however, this may be truly attributable to strain differences, since different strains were concerned in all cases where differences appear. It is worthy of note, however, that galactose is slowly fermented (Johnson, 1935, and Table 3 of this report) and acid production is not high, particularly with *T. geleii* H. The high buffer concentration and short incubation time used by Elliott (1935*c*) could account for his failure to observe fermentation of galactose.

Since it cannot be stated with certainty that Johnson's (1935) strain called "*Glaucoma piriformis*" (Hetherington strain) is the same as the "*Colpidium campylum*" isolated by Hetherington (1933) and later called "*Glaucoma pyriformis*" (Hetherington, 1934), it is possible that the difference found by Johnson and by Elliott in mannose fermentation is due to strain difference. This may also apply to galactose fermentation. The disagreement with regard to lactose may again be due to a strain difference. These, then, are not discrepancies but serve to indicate the danger of assuming that all strains of *T. geleii* react similarly. That there are obvious differences between strains is apparent from the data presented here. On the other hand, reports on *T. geleii* W with cellobiose differ. The only difference in the medium used (Kidder, 1941*b*, and the present report) is in the concentration of the proteose-peptone. The earlier results were obtained with a medium containing 2 per cent proteose-peptone, the present results with 1 per cent.

One other phenomenon in connection with carbohydrate fermentation is worthy of note. Those tubes containing a nonfermentable carbohydrate in all cases became markedly alkaline (above the limit of the indicator). This is presumably due to the production of ammonia as a product of nitrogen metabolism. It is this ammonia production which is, no doubt, responsible for the reversal of the reaction of the medium after *T. geleii* H has fermented starch. Certain other strains showed this effect to a less marked degree (only after 3 weeks' incubation) with the polysaccharides and some of the monosaccharides. The speed with which the change from acid back to neutral or alkaline occurs appears to be related to the sensitivity of the organism to acid or to some other condition in old cultures. Those which are least sensitive are able to maintain a high population level for sufficiently long periods of time eventually to produce enough ammonia to reverse the reaction of the medium when the carbohydrate has been exhausted. A rough calculation from the data presented by Loefer (1938) shows that the carbohydrate would be completely utilized by *T. geleii* H long before 7 days, assuming a population density maintained at approximately 100,000 organisms per milliliter during that period. These characteristics of *T. geleii* H (acid insensitivity and high ammonia production) may account for the results with galactose, which is fermented very slowly.

From our results we believe that the simplest medium to use as a base for carbohydrate tests is 1 per cent proteose-peptone. We suggest, therefore, that for diagnostic purposes 1 per cent proteose-peptone containing 0.002 per cent brom

thymol blue (because of its comparatively low toxicity) and 0.5 per cent of the carbohydrate to be tested be used (in the earlier report [Kidder, 1941*b*] the concentration of brom thymol blue was erroneously given as 0.02 per cent). The cultures should be incubated for 2 weeks at 25.5° C. and readings made at 5, 7, and 14 days. Differences between strains have been consistently obtained under these conditions, and later work with the same technique should succeed in placing new strains in their proper relationship to those already established.

The fallacies inherent in the method of omission of single amino acids from a complete diet as a means of determining amino acid requirements have been pointed out (Gladstone, 1939; Hegsted, 1944; Kidder and Dewey, 1945*a*). Nevertheless, such experiments are useful as a preliminary step in the elucidation of requirements. As more evidence is accumulated, it becomes apparent that the metabolism of amino acids is more complex than originally believed. There are mutual antagonisms and interdependencies that are not revealed by such simple procedures. The results reported here with glycine are a case in point. According to the generally accepted criteria, this amino acid, as well as the basic ten, would have to be considered indispensable for all the strains studied. Nevertheless, only two of the strains were able to grow normally in the mixture of the ten plus glycine, while others showed definite inhibition in the presence of glycine. On the other hand, serine would have to be regarded as a dispensable amino acid for all the strains but the two which refused to grow normally in the complete medium. Yet all but two of the strains gave no growth in the mixture of the basic ten amino acids, and all gave excellent growth in the mixture of ten plus serine. On the basis of these results, the conclusion must be drawn that serine is an essential amino acid. In the case of the two strains of *T. vorax*, however, serine can be replaced by glycine. The question then arises as to which is to be considered the essential amino acid. In some cases cystine appears to be able to substitute for serine to some extent, but here the structural similarity of the two amino acids is readily apparent as an explanation.

In view of the stimulatory, or even essential, nature of what at first appeared to be dispensable amino acids, it was of great importance to retest the indispensability of the basic ten amino acids in the presence of cystine, serine, and glycine or combinations of these three, omitting the other dispensable amino acids. The results of these experiments indicate that *T. geleii* E, T-P, and GHH may be able to synthesize slowly certain of the amino acids in the essential group. The most striking effect, however, was the fact that all strains except *T. vorax* V gave growth in the absence of valine, provided that cystine, glycine, and serine were present. In most cases glycine and serine were sufficient to give this effect and in others cystine and serine or cystine and glycine. Only *T. geleii* E and GHH grew with the addition of only serine when valine was omitted. Somewhat similar, but less striking, effects were obtained in the case of threonine. It is probable that, by adjusting the concentrations of the amino acids, more clear-cut evidence of synthetic capacity could be demonstrated for all strains. In the light of the results reported by Gladstone (1939) for *Bacillus anthracis*, the importance of the concentration of amino acids present is readily seen. It is evident that in the case of *Tetrahymena* we also are dealing with inhibitory effects and release of inhibition such as were described using valine, leucine,

isoleucine, threonine, and serine. If *Tetrahymena* is comparable to *B. anthracis*, the effectiveness of serine in promoting growth may be due to a release of inhibition by valine and isoleucine. The occurrence of inhibition appears to be widespread among microörganisms (Porter and Meyers, 1944; Nielsen and Hartelius, 1938; Doerman, 1944; Snell and Guirard, 1943), and the question of such interactions has been raised by the work of Hegsted (1944) on the chick.

Other examples of inhibition and release are observed when certain of the dispensable amino acids are added to the basic ten or combinations of the ten with the stimulatory amino acids. Alanine in this case is seen to be inhibitory, the inhibition being released only by combinations of the stimulatory amino acids. This is in direct contrast to the effects of alanine described by Snell and Guirard (1943) for *Streptococcus lactis* R. Under their experimental conditions alanine caused stimulation of growth and a release of inhibition produced by glycine. According to the single-omission technique, *S. lactis* R requires both alanine and glycine. Further work in proving the essential nature of these amino acids would be of great interest.

Besides that given by alanine, the most marked inhibitory effect was that produced by tyrosine. In this case, however, the strains of *T. vorax* were much less affected than the strains of *T. geleii*. It would appear that the aliphatic amino acids are chiefly concerned in the inhibitory effects described above and in the literature, although Doerman (1944) describes a mutual antagonism between arginine and lysine for a "lysineless" mutant of *Neurospora*. The general explanation for these effects is the blocking of enzymes by an excess of a compound resembling in chemical structure the specific substrate for the enzyme. Release is produced when an excess of the specific substrate is present. The structural similarity of the aliphatic amino acids needs no additional comment here.

On the basis of the data on growth in amino acid mixtures so far reported, the eight strains of *Tetrahymena* fall into three groups. The two strains of *T. vorax* are readily separated from the others by the fact that they are greatly stimulated by the addition of glycine to the mixture of the basic ten amino acids. It is on the basis of this reaction, as well as on the fermentation of lactose, that strain PP was designated as *T. vorax*. The two strains of *T. vorax* differ in the greater sensitivity of strain V to cystine inhibition and its apparent inability to synthesize valine in the presence of the stimulatory amino acids.

The remaining six strains of *Tetrahymena* fall into two groups of three. *T. geleii* H, T, and W constitute one group; *T. geleii* E, T-P, and GHH the other. This separation rests mainly upon the sensitivity of the latter group to certain amino acids, which seems to be correlated with greater synthetic powers. *T. geleii* GHH resembles the other group of *T. geleii* in that it is less sensitive to inhibitory effects than the others in its own group. It appears, however, to have the most marked synthetic ability of any of the strains. *T. geleii* T-P is separated from *T. geleii* E by its stimulation by cystine and by its inability to ferment galactose.

Of the three other strains, *T. geleii* W is marked by its ability to grow in only ten amino acids and *T. geleii* H by its ability to ferment galactose very slowly, as well as by the reversal of reaction after fermentation of starch.

There is one other difference, not heretofore mentioned, among these strains, which is quite obvious when a series of tube cultures in 1 per cent pro-

teose-peptone is held up to the light. It is then seen that certain of the strains cause a purplish coloration of the medium. This coloration is most marked in *T. geleii* T. In decreasing order of color production are *T. geleii* T, H, T-P, W, and GHH. In the last two color production is very slight, and in *T. geleii* E it is absent. The two strains of *T. vorax* produce no color.

Thus it may be seen that there are many similarities between the strains of *T. geleii* and those of *T. vorax*, but none of them are biochemically identical (see Table 23). These similarities and differences follow those found in preliminary serological investigations using cross-adsorption techniques.

TABLE 23

SUMMARY OF STRAIN DIFFERENCES

Strain	Galactose	Lactose	Starch	19 Amino Acids	10 Amino Acids	Stimulation by		Valine Synth.	Color Prod.
						Glycine	Cystine		
H	Slow	−	+ Reversal	+	−	−	−	+	Moderate
E	+	−	+	−	−	−	−	+	None
T-P	−	−	+	−	−	−	+	+	Moderate
T	−	−	+	+	−	−	−	+	Strong
W	−	−	+	+	+	−	−	+	Weak
GHH	−	−	+	+	±	±	±	+	Weak
V	+	+	+	+	−	+	−	−	None
PP	+	+	+	+	−	+	−	±	None

SUMMARY

1. The sources of six strains of *T. geleii* and two strains of *T. vorax* are discussed and the literature concerning them summarized.

2. The ability of these strains to ferment 27 carbohydrates was tested and a diagnostic system proposed on the basis of the reactions in galactose, lactose, and starch.

3. Preliminary studies of amino acid requirements indicate that arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine may be regarded as essential for all strains.

4. Only the two strains of *T. vorax* gave normal growth in a mixture of the above-mentioned amino acids.

5. Replacement of glycine by serine permitted growth of all strains.

6. *T. geleii* E and T-P failed to give normal growth in a mixture of nineteen amino acids due to inhibitory effects of some of the amino acids.

7. In the presence of the stimulatory amino acids (cystine, glycine, and serine) certain of the essential amino acids seem to be synthesized.

8. The dispensable amino acids (alanine, aspartic acid, glutamic acid, hydroxyproline, proline, and tyrosine) are, in general, inhibitory.

9. Inhibition by the dispensable amino acids may be released by the stimulatory amino acids either singly or in combination.

10. None of the eight strains of ciliates studied require thiamine.

11. The strains differ in the intensity of the purplish coloration of the medium (1 per cent proteose-peptone) produced.

12. All strains differ either in carbo-

hydrate fermentation or in reaction to amino acids.

13. Larger differences are found between the six strains of *T. geleii* than between the two strains of *T. vorax* (Table 23).

LITERATURE CITED

BAKER, E. G. S., and BAUMBERGER, J. P. 1941. The respiratory rate and the cytochrome content of a ciliate protozoan (*Tetrahymena geleii*). Jour. Cell. and Comp. Physiol., **17**:285–304.

BARBER, M. A. 1944.The rearing of sterile adult *Anopheles*. Pub. Health Reports, **59**:1384–87.

BOND, R.M. 1933. A contribution to the study of the natural food cycle in aquatic environments, with particular consideration of microörganisms and dissolved organic matter. Bull. Bingham Oceanogr. Coll., **4**, No. 4, 1–89.

BROWN, M. G. 1940. Growth of protozoan cultures. II. *Leucophrys patula* and *Glaucoma pyriformis* in a bacteria-free medium. Physiol. Zoöl., **13**: 277–82.

CLAFF, C. L. 1940. A migration dilution apparatus for the sterilization of protozoa. Physiol. Zoöl., **13**:334–41.

CLAFF, C. L.; DEWEY, V. C.; and KIDDER, G. W. 1941. Feeding mechanisms and nutrition in three species of *Bresslaua*. Biol. Bull., **81**:221–34.

COLAS-BELCOUR, J., and LWOFF, A. 1925. L'Utilization des glucides par quelques protozoaires. Compt. rend. Soc. biol., **93**:1421–22.

DEWEY, V. C. 1941. The nutrition of *Tetrahymena geleii* (Protozoa, Ciliata). Proc. Soc. Exper. Biol. and Med., **46**:482–84.

———. 1944. Biochemical factors in the maximal growth of *Tetrahymena*. Biol. Bull., **87**:107–20.

DOERMAN, A. H. 1944. A lysineless mutant of *Neurospora* and its inhibition by arginine. Arch. Biochem., **5**:373–84.

ELLIOTT, A. M. 1933. Isolation of *Colpidium striatum* Stokes in bacteria-free cultures and the relation of growth to the pH of the medium. Biol. Bull., **65**:45–56.

———. 1935*a*. The influence of pantothenic acid on the growth of protozoa. *Ibid.*, **68**:82–92.

———. 1935*b*. Effects of certain organic acids and protein derivatives on the growth of *Colpidium*. Arch. f. Protistenk., **84**:472–94.

———. 1935*c*. Effect of carbohydrates on growth of *Colpidium*. *Ibid.*, pp. 156–74.

———. 1938. The influence of certain plant hormones on the growth of protozoa. Physiol. Zoöl., **11**:31–39.

———. 1939. The vitamin B complex and the growth of *Colpidium striatum*. *Ibid.*, **12**:363–73.

FURGASON, W. H. 1936. A contribution to the morphology and taxonomy of some holotrichous ciliates. Dissertation. Stanford University Library.

———. 1940. The significant cytostomal pattern of the "*Glaucoma-Colpidium* group," and a proposed new genus and species, *Tetrahymena geleii*. Arch. f. Protistenk., **94**:224–66.

GIESE, A. C. 1938. Differential susceptibility of a number of protozoans to ultraviolet radiations. Jour. Cell. and Comp. Physiol., **12**:129–38.

GLADSTONE, G. P. 1939. Inter-relationships between amino acids in the nutrition of *B. anthracis*. Brit. Jour. Exper. Path., **20**:189–200.

GLASER, R. W., and CORIA, N. A. 1935. The culture and reactions of purified protozoa. Amer. Jour. Hyg., **21**:111–20.

HALL, R. H. 1938. The oxygen-consumption of *Colpidium campylum*, Biol. Bull., **75**:395–408.

HALL, R. P. 1938. Effects of manganese on the growth of *Euglena anabaena*, *Astasia* sp., and *Colpidium campylum*. Arch. f. Protistenk., **90**:178–84.

———. 1939. Pimelic acid as a growth stimulant for *Colpidium campylum*. *Ibid.*, **92**:315–19.

———. 1940. Riboflavin and thiamin as growth-factors for the ciliate, *Colpidium campylum*. Anat. Rec., Suppl. **78**:164.

———. 1942. Incomplete proteins as nitrogen sources, and their relation to vitamin requirements in *Colpidium campylum*. Physiol. Zoöl., **15**:95–107.

HALL, R. P., and ELLIOTT, A. M. 1935. Growth of *Colpidium* in relation to certain incomplete proteins and amino acids. Arch. f. Protistenk., **85**:443–50.

HALL, R. P., and LOEFER, J. B. 1940. Effects of culture filtrates and old medium on growth of the ciliate *Colpidium campylum*. Proc. Soc. Exper. Biol. and Med., **43**:128–33.

HALL, R. P., and SHOTTENFELD, A. 1941. Maximal density and phases of death in populations of *Glaucoma piriformis*. Physiol. Zoöl., **14**:384–93.

HEGSTED, D. M. 1944. Growth in chicks fed amino acids. Jour. Biol. Chem., **156**:247–52.

HEGSTED, D. M., and WARDWELL, E. D. 1944. On the purity of synthetic *dl*-leucine. Jour. Biol. Chem., **153**:167–70.

HETHERINGTON, A. 1933. The culture of some holotrichous ciliates. Arch. f. Protistenk., **80**:255–80.

———. 1934. The rôle of bacteria in the growth of *Colpidium colpoda*. Physiol. Zoöl., **7**:618–41.

———. 1936. The precise control of growth in the pure culture of a ciliate, *Glaucoma pyriformis*. Biol. Bull., **70**:426–40.

JAHN, T. L. 1936. Effect of aeration and lack of CO_2 on the growth of bacteria-free cultures of protozoa. Proc. Soc. Exper. Biol. and Med., **33**:494–98.

JOHNSON, D. F. 1935. Fermentation of carbohydrates by *Glaucoma* and effects of carbo-

hydrates on growth of two species. Anat. Rec., Suppl. 64:106–7.

———. 1936. Isolation of *Glaucoma ficaria* Kahl in bacteria-free cultures, and growth in relation to pH of the medium. Arch. f. Protistenk., 86: 263–77.

JOHNSON, W. H., and BAKER, E. G. S. 1943. Effects of certain B vitamins on populations of *Tetrahymena geleii*. Physiol. Zoöl., 16:172–85.

KIDDER, G. W. 1939*a*. The effect of biologically conditioned medium upon the growth of *Colpidium campylum*. Biol. Bull., 77:297–98.

———. 1939*b*. The effect of biologically conditioned medium on the growth rate and population yield of certain ciliated protozoa. Science, 90:405.

———. 1941*a*. Growth studies on ciliates. V. The acceleration and inhibition of ciliate growth in biologically conditioned medium. Physiol. Zoöl., 14:209–26.

———. 1941*b*. Growth studies on ciliates. VII. Comparative growth characteristics of four species of sterile ciliates. Biol. Bull., 80:50–68.

KIDDER, G. W., and DEWEY, V. C. 1942. The biosynthesis of thiamine by normally athiaminogenic microörganisms. Growth, 6:405–18.

———. 1944. Thiamine and *Tetrahymena*. Biol. Bull., 87:121–33.

———. 1945*a*. Studies on the biochemistry of *Tetrahymena*. I. Amino acid requirements. Arch. Biochem., Vol. 6. (In press.)

———. 1945*b*. Studies on the biochemistry of *Tetrahymena*. II. Factor three. *Ibid.*

KIDDER, G. W.; LILLY, D. M.; and CLAFF, C. L. 1940. Growth studies on ciliates. IV. The influence of food on the structure and growth of *Glaucoma vorax* sp. nov. Biol. Bull., 78:9–23.

KIDDER, G. W., and STUART, C. A. 1939. Growth studies on ciliates. I. The role of bacteria in the growth and reproduction of *Colpoda*. Physiol. Zoöl., 12:329–40.

KLINE, A. P. 1943. The nitrogen compounds necessary for growth in *Colpidium striatum* Stokes, with special reference to the amino acids. Physiol. Zoöl., 16:405–17.

LILLY, D. M. 1942. Nutritional and supplementary factors in the growth of carnivorous ciliates. Physiol. Zoöl., 15:146–67.

LOEFER, J. B. 1938. The utilization of dextrose by *Colpidium*, *Glaucoma*, *Chilomonas*, and *Chlorogonium* in bacteria-free cultures. Jour. Exper. Zoöl., 79:167–83.

LOEFER, J. B., and HALL, R. P. 1936. Effect of ethyl alcohol on the growth of eight protozoan species in bacteria-free cultures. Arch. f. Protistenk., 87:123–30.

MARBARGER, J. P. 1943. The production of growth substances by *Colpidium striatum* (Stokes). Physiol. Zoöl., 16:186–98.

NIELSEN, N., and HARTELIUS, V. 1938. Wuchsstoffwirkung der Aminosäuren. III. Untersuchungen über die Wuchsstoffwirkung von β-alanin, β-alanylglycin, Asparaginsäure, Glycyl-Asparaginsäure und verwandte Stoffe auf Hefe. Compt. rend. lab. Carlsberg (Sér. physiol.), 22:271–80.

ORMSBEE, R. A. 1942. The normal growth and respiration of *Tetrahymena geleii*. Biol. Bull., 82: 423–37.

PETERSON, R. E. 1942. Essential factors for the growth of the ciliate protozoan, *Colpidium campylum*. Jour. Biol. Chem., 146:537–45.

PHELPS, A. 1935. Growth of protozoa in pure culture. I. Effect upon the growth curve of the age of the inoculum and of the amount of the inoculum. Jour. Exper. Zoöl., 70:109–30.

———. 1936. Growth of protozoa in pure culture. II. Effect upon the growth curve of different concentrations of nutrient materials. *Ibid.*, 72:479–96.

PORTER, J. R., and MEYERS, F. P. 1944. Nutrition of *Proteus morganii:* amino acid interrelationships. Jour. Bact., 47:29–30.

ROSE, W. C. 1938. The nutritional significance of the amino acids. Physiol. Rev., 18:109–36.

SNELL, E. E., and GUIRARD, B. M. 1943. Some interrelationships of pyridoxine, alanine and glycine in their effects on certain lactic acid bacteria. Proc. Nat. Acad. Sci., 29:66–73.

TANZER, C. 1941. Serological studies with living protista. Jour. Immunol., 42:291–312.

TAYLOR, C. V.; THOMAS, J. O.; and BROWN, M. G. 1933. Studies on the protozoa. IV. Lethal effects of the X-radiation of a sterile culture medium for *Colpidium campylum*. Physiol. Zoöl., 6:467–92.

THOMAS, J. O. 1943. The anaerobic carbohydrate metabolism of *Tetrahymena geleii*. Abstr. Diss. Stanford Univ., 18:20–25.

VAN NIEL, C. B.; THOMAS, J. O.; RUBEN, S.; and KAMEN, M. D. 1942. Radioactive carbon as an indicator of carbon dioxide utilization. IX. The assimilation of carbon dioxide by protozoa. Proc. Nat. Acad. Sci., 28:157–61.

A SIMPLE METHOD FOR DIVISION-RATE DETERMINATION IN PARAMECIUM

ARTHUR C. GIESE
School of Biology, Stanford University, California

A SIMPLE culture method has been used successfully for the determination of the division rate of three species of *Paramecium*—*P. aurelia*, *P. caudatum*, and *P. multimicronucleatum*—and also for *Blepharisma undulans* in a number of experimental studies (Giese, 1938, 1939*a*, *b*). The method is therefore briefly described below in order to make it available to others.

From a good wild culture of the desired organism, select a few vigorous specimens and wash free of bacteria by the method of Parpart (1928). This is done by picking up the paramecia in a sterile *inoculation pipette* and transferring to a sterile watch glass containing ½ cc. of sterile balanced salt solution. After a few minutes this is repeated three more times, with fresh sterile pipettes and dishes each time. The paramecia are allowed to swim in the fourth watch glass for several hours, and the process is again repeated four times, allowing the paramecia to remain in the eighth watch glass overnight to defecate the spores which may be contained inside the food vacuoles (Luck, Sheets, and Thomas, 1931). On the following morning they are given four more washes and transferred to the culture medium inoculated with the desired strain of bacteria, to be described below.

The watch glasses found especially convenient are Bureau of Plant Industry Syracuse watch glasses, which hold about 1 cc. of solution. These are generally placed in groups of four per Petri dish, and to each is added about ½ cc. of the balanced medium. These dishes, as well as all others mentioned, are sterilized at 15 pounds pressure for 15–20 minutes in an autoclave or pressure cooker.

The balanced medium consists of 6 per cent solutions of NaCl, KCl, $CaCl_2$, $MgCl_2$, and $MgSO_4$ in the proportions: 100, 2.2, 1.0, 7.8, and 3.8, respectively, diluted five hundred times. Sea water diluted two hundred and fifty times may be used, and pond water will do. Sometimes ordinary distilled water is toxic and must be redistilled in a still free of copper parts and of toxic volatile materials.

The *inoculating pipettes* used in the procedure may be made by drawing out a 12-cm. piece of 4-mm.[1] soft glass tubing. The pipette tip is cut to the desired length by scratching with a diamond pencil or a crystal of carborundum and pressing lightly. The fine tip (about 0.2 mm.) can be fire-polished by quickly drawing through the Bunsen flame. The broad end is plugged with cotton, and the pipettes are placed, tip down, in a long test tube containing a cotton wad at the bottom to protect the fine tips. The test tube is then plugged and sterilized.

Lettuce infusion is used for growing the paramecia, although hay, hay-wheat, mushroom, and other infusions have been used as well. A 0.5-gm. sample of lettuce powder is added to 50 cc. of distilled water (or pond water) and boiled for a minute or so. To this is added 850 cc. of distilled water and 100 cc. of Sorensen buffer at pH 7.0. The infusion is stirred,

[1] All diameters given are inside diameters.

and 15–20-cc. samples are withdrawn and poured into test tubes. These are plugged and autoclaved. Such samples will keep indefinitely except that the water evaporates.

The Sorensen buffer of approximately pH 7.0 is made up by mixing 3.8 parts of 0.5 M KH_2PO_4 (Sorensen) with 6.2 parts of 0.5 M Na_2HPO_4 and diluting tenfold. For accurate work it is necessary to test the pH and adjust the stock buffer by additions of the appropriate salts.

The lettuce powder used in making the infusion is prepared as follows: The outer leaves of a head of lettuce are discarded, but the inner leaves are washed and wiped. They are then dried over an electric hotplate on a glass griddle or in an oven if no volatile toxic materials have been used in it. When the leaves are crisp and dry, they can be ground with a mortar and pestle. The powder will keep indefinitely if dry. Burned or browned leaves should not be used.

About 24 hours before use, the lettuce infusion is inoculated with a loopful of a strain of bacteria found suitable. In this laboratory *Pseudomonas ovalis* isolated from a vigorous lettuce infusion has been found satisfactory as food for *Paramecium*. This form can be easily grown on 2 per cent agar containing 2 per cent yeast extract. The bacteria should be transferred weekly, as old cultures show variants of unsatisfactory nature.

The single washed paramecium is pipetted into a small portion of bacterized lettuce infusion contained in a small culture tube made in the following way: The 3-mm. soft glass tubing is cut into approximately 6.5-cm. lengths, and one end is heated until it closes. The other end is heated and the mouth widened by twirling on a pointed carbon inserted into the opening. When cool, the tube may be washed by inserting a pipette reaching to the bottom and blowing in distilled water from a wash bottle. The water may be emptied by a quick shake. The tubes are plugged by inserting firm conical cotton plugs and are then autoclaved. When a bacterized infusion is added with aseptic precautions, only one strain of bacteria will be present in the tube. The *transfer pipettes* used for adding infusion to the culture tubes should be larger than those for inoculating paramecia. A piece of glass tubing about 16 cm. long may be used to make two such pipettes. A convenient size of pipette has drawn and undrawn portions each 6.5 mm. long, with a tip diameter of about 0.4 mm. The pipettes are plugged, placed in test tubes, and sterilized.

The culture tubes of the type described above are convenient because they can be laid on the stage of a dissecting microscope and the number of paramecia counted easily, at least until 32 have developed. If the paramecia are removed with a pipette, even a larger number can be counted accurately. The medium does not evaporate easily from such a tube, and the temperature can be adjusted quickly by immersing the tubes in a water bath at the desired temperature. By grinding, on an emery wheel, a portion of the tube just under the mouth, a ground-glass surface for pencil labeling is easily made. A convenient rack for the tubes is a 50-ml. beaker. The whole method has the advantage of minimal expense.

Once a good culture of paramecia free of bacteria other than the strain used for food (as can be determined by streaking out the infusion and observing the colonies on a plate or by plating the final washes according to Parpart [1928]) has been secured, if aseptic precautions are observed, there is no need for further washing of the paramecia; and each time they can be transferred directly from a good culture to a fresh culture tube.

To give some idea of the utility, flexibility, and variability of the method with variation of the environmental factors, a number of graphs are introduced, since these summarize the data most clearly and concisely. In Figure 1, *A* and *B*, is

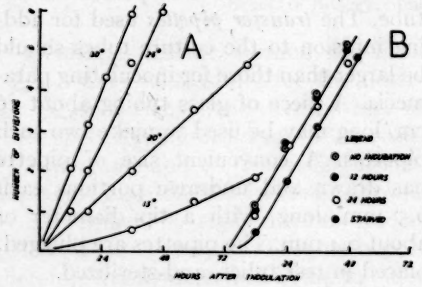


FIG. 1.—(*A*) Effect of temperature on division rate of *P. multimicronucleatum*. (*B*) Effect of starvation of *P. caudatum* and incubation of medium after inoculation with bacteria on division rate.

shown a typical curve for division of paramecia transferred from a culture in the logarithmic phase. There is no lag in division, and a straight line may be drawn through the points. When the food supply is relatively exhausted, the curve flattens out. If paramecia are withdrawn during the logarithmic phase and transferred to a freshly prepared culture tube, a straight line is again obtained without a lag. If the paramecia are allowed to starve for 24 hours or more in balanced salt medium before transfer, a lag is observed, as in Figure 1, *B*. The effect of starvation is shown in Figure 2, *B*.

In Figure 1, *B*, are shown the effects of different periods of incubation of the bacteria before the inoculation of paramecia. The bacteria may be introduced just at the time paramecia are inoculated, and no difference in division of paramecia is observed; but it is not desirable to do this, for the following reason: If a contaminant were to enter at the time paramecia are added and if a large quantity of nutrient were still available for the bacteria, the contaminant would multiply far more than in a medium already occupied by *Pseudomonas ovalis*. Short periods might be used only if it is not possible to inoculate ahead.

Another variable to be considered is temperature. Results for *Paramecium multimicronucleatum* are shown in Figure 1, *A*. In these studies the bacteria were inoculated in the medium 48 hours before the paramecia were added, and the test tubes were kept at 26° C. for that time. Then the small culture tubes were filled from these and placed at the appropriate temperatures, at which time the paramecia were inoculated. Under the conditions here used, the strains of *P. caudatum* were more sensitive to higher temperatures than strains of either *P. multimicronucleatum* or *P. aurelia*.

In Figures 2 and 3 are shown the effects of three other variables: pH, buffer concentration, and buffer type. Ordi-

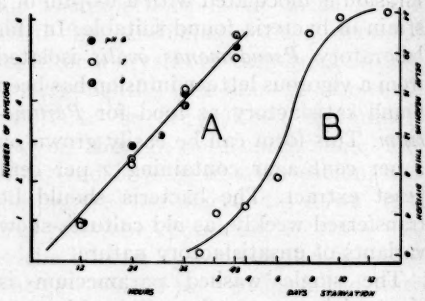


FIG. 2.—(*A*) Effect of pH on division rate. The values given are initial values. In two other sets of experiments the division rates at different pH values were not so close as in this series (*P. multimicronucleatum*). (*B*) Hours delay in reaching the third division after inoculating *P. caudatum*, starved for the indicated period of time in balanced salt solution, into medium with bacteria.

narily, a buffer concentration of 0.005 M is used, but this does not hold the pH completely constant, variation of a few

tenths of a pH unit being found. However, a higher concentration of buffer is undesirable, since the rate of division is decreased, as shown in the figure. When the initial pH is set at 6, 7, or 8, there is no difference in the rate of division, provided that the bacteria are allowed to develop in the absence of buffer and the buffer is added just before the addition of paramecia, as shown in the curves in Figure 3. Both buffers made from NaH_2PO_4—Na_2HPO_4 and buffers of KH_2PO_4—Na_2HPO_4 are satisfactory, the rate of division being the same. A buffer made completely from potassium phosphates is so toxic that few divisions occur.

Another variable tested was the concentration of lettuce infusion. If double the amount of dry lettuce is used, the medium is satisfactory; but the division rate of paramecia remains the same as at the lower concentration, and the only advantage gained is the greater population of bacteria. Since it is impossible to count accurately over 32 paramecia per tube and since a straight line is usually obtained up to six divisions for the concentration described, there is no advantage in an increase. Half the concentration will do, but there is a decline in rate before the sixth division.

The main disadvantage of this method, compared to a mass culture method (e.g., Giese and Taylor, 1935), is the fact that only a relatively small number of animals is followed. On the other hand, the accuracy with which the individual events can be determined has much in its favor over the determination on a large sample in a mass culture.

SUMMARY

A method for isolation culture of *Paramecium aurelia*, *P. caudatum*, *P. multimicronucleatum*, and for *Blepharisma undulans* is described. The culture tubes and transfer pipettes are easily made of glass tubing. The method has the advantages of making easy and accurate determinations of division rate. The effects of variations in temperature, concentration of nutrient, buffer type and concentration, pH, and type of inoculum are recorded in graphs.

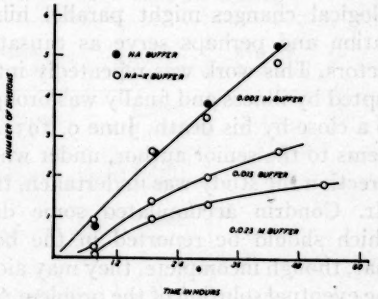


FIG. 3.—Effect of buffer type and buffer concentration on division rate (*P. multimicronucleatum*).

LITERATURE CITED

GIESE, A. C. 1938. Size and conjugation in *Blepharisma*. Arch. f. Protistenk., **91**:125–34.

———. 1939*a*. Ultraviolet radiation and cell division: effects of λ 2654 and 2804 A upon *Paramecium caudatum*. Jour. Cell. and Comp. Physiol., **13**:139–50.

———. 1939*b*. Studies on conjugation in *Paramecium multimicronucleatum*. Amer. Nat., **73**:432–44.

GIESE, A. C., and TAYLOR, C. V. 1935. Paramecia for experimental purposes in controlled mass cultures on a single strain of bacteria. Arch. f. Protistenk., **84**:225–31.

LUCK, J. M.; SHEETS, G.; and THOMAS, J. O. 1931. The role of bacteria in the nutrition of protozoa. Quart. Rev. Biol., **6**:46–58.

PARPART, A. K. 1928. The bateriological sterilization of *Paramecium*. Biol. Bull., **55**:113–20.

PHYSIOLOGICAL STUDIES ON HIBERNATION IN THE CHIPMUNK

ALVALYN E. WOODWARD AND JOHN M. CONDRIN[1]

IN THE spring of 1932 the junior author began a study of the seasonal activities of the eastern chipmunk, *Tamias striatus lysteri* (Richardson) to determine, first, whether or not it hibernates and, second, what, if any, physiological changes might parallel hibernation and perhaps serve as causative factors. This work was repeatedly interrupted by illness and finally was brought to a close by his death, June 9, 1937. It seems to the senior author, under whose direction the study was undertaken, that Mr. Condrin accumulated some data which should be reported in the hope that, though incomplete, they may aid in the eventual solution of the problem. She cannot, however, assume responsibility for the accuracy of these results.

The question as to whether chipmunks hibernate in the vicinity of Toledo, Ohio, was partially answered by the fact that, in four years' collecting, none were seen between late November and late February (Condrin, 1936). Additional evidence is furnished by the observation of spontaneous hibernation in some of the animals carried throughout the winter in the laboratory. Chipmunk No. 70, for example, was found, December 22, curled up in the bedding in one corner of the cage in deep torpor (room temperature 16° C.). Its rectal temperature was 22°, respiration 6/min., pulse 170/min., as compared to normal respiration of about 150/min. and pulse of about 250/min.

Various vital processes and organs of chipmunks were studied throughout the year to learn whether there were seasonal changes in physiology or histology that could throw light on the factors leading to or conditioning hibernation.

Blood counts.—The blood counts made on twelve active animals (Table 1) show a tendency toward high erythrocyte count in August, followed by decreasing values in the fall. However, while the number of animals from which blood was taken compares favorably with those in other studies, it is small, and the wide variations for the month of August forbid our drawing any definite conclusion. The white-cell counts are even more erratic. The high values for animals Nos. 99 and 104 seem distinctly above the normal range, and there is little indication of seasonal variation. Rasmussen (1916) found still greater irregularity in the leucocyte numbers in woodchucks. His data show, however, an interesting increase in erythrocytes, specific gravity, and hemoglobin content of the blood during dormancy, followed by a sudden decrease immediately upon waking. After his animals were fed, the blood became still more dilute. Dubois (1896) and Polimanti (1912) are quoted by Rasmussen as having found similar changes in the blood of marmots upon awakening. Likewise, Worth (1932) found that in bats the red-cell counts were 22 per cent higher and the leucocyte counts 200 per cent higher in winter (semidormancy) than in summer. We have no explanation for the discrepancy between these results and ours.

Adrenal weights.—In another phase of the study, endocrine glands were removed from a series of animals throughout the year, weighed, and then fixed for section-

[1] Contribution from the Department of Zoölogy, University of Michigan, Ann Arbor, Michigan.

ing. A definite seasonal change in size was found in the pituitaries (data lost) and adrenals. The weight of the latter (Table 2 and Fig. 1) increased from an average of 7.7 gm. in January to a high level, about 21.5 gm., extending from April to September, then slowly declined during the autumn and early winter to a January low. It should be noted that this curve with its plateau is of quite different shape from that found by Zalesky (1934) for *Citellus tridecemlineatus*. In the latter animal the weight of the adrenal increases from a December minimum to a sharply defined maximum in May, then regresses. In this connection one should recall the difference in the reproductive cycles of the two animals. *Citellus* breeds but once a year, in the spring, while the chipmunk (Condrin, 1936) apparently has two litters a year, with the reproductive peaks in April and July. The similarity between the curves of reproduction and of adrenal size is readily understood when one recalls that the pituitary is said to "control" both the adrenals and the gonads.

TABLE 1

BLOOD COUNTS

Month	Animal	Red Blood Cells		White Blood Cells	
		Individual	Average for Month	Individual	Average for Month
January	97	7,760,000	6,780,000	3,800	3,220
	97	5,800,000		2,640	
February	99	5,776,000	5,588,000	13,140	8,970
	7	5,400,000		4,800	
March	101	9,256,000	8,228,000	3,600	3,100
	102	7,200,000		2,600	
April	98	7,120,000	.....	.....	.....
August	83*	13,400,000	10,748,000	4,400	4,147
	85	12,200,000		4,000	
	87	6,744,000		4,040	
September	91	5,248,000	.....	6,700	.....
October	92	5,360,000	.....	6,900	.....
November	104	2,560,000	.....	14,500	.....

* Two days postpartum.

TABLE 2

WEIGHTS OF ADRENAL GLANDS

Month	No. Glands Weighed	Average Weight (Gm.)
January	2	7.7
February	4	12.5
March	2	12.5
April	2	18.75
May	2	18.5
June	.....	.....
July	4	21.6
August	18	21.5
September	6	21.3
October	10	15.8
November	6	12.2
December	4	13.65

Blood sugar.—In a third line of investigation, the junior author carried out a series of determinations of fasting blood sugar, using the micromethod of Folin and obtaining blood from the heart. These show a striking seasonal variation even in the animals at room temperature (Table 3 and Fig. 1). The highest average value—180 mg./100 cc.—occurred in July

and the lowest—100 mg./100 cc.—in January, with a distinct decrease, about 20 per cent, in the autumn before time for hibernation. In twelve chipmunks, Mr. Condrin was able to make repeated determinations of blood sugar at intervals

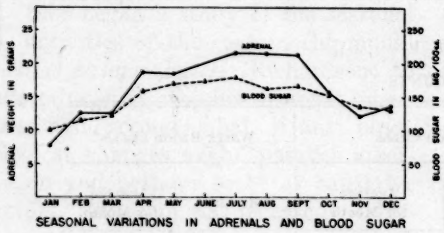

ADRENAL WEIGHT IN GRAMS
BLOOD SUGAR IN MG./100cc.
ADRENAL
BLOOD SUGAR
JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC
SEASONAL VARIATIONS IN ADRENALS AND BLOOD SUGAR

FIG. 1

of two or more weeks. A few animals survived as many as six such tests. In these individuals the blood sugar varied according to the normal curve.

While, as stated, this seasonal change in blood sugar occurs at room temperature, a marked reduction may be found even in midsummer if chipmunks are put into the refrigerator at a temperature of 0°–6° C. By referring to Table 4 it will be noted that the decrease of blood sugar varied greatly in this experiment. If, moreover, the amount present dropped to 100 mg./100 cc., the animal became torpid; on the other hand, animal No. 25, with a blood sugar of 112 mg./100 cc. was only semitorpid. While these data are too meager to permit any conclusion as to a critical sugar level at which a chipmunk might be expected to become dormant, they show clearly the depressing effect of cold on blood sugar.

Temperature.—Like other hibernating mammals (Benedict and Lee, 1938), chipmunks are only partially homoiothermic. Their body temperatures, while active, range between 35.8° and 39° C., with an average of about 37.5°. They were unable to maintain this when put into the refrigerator (Table 4), where rectal temperatures of 16.8°–29° were found in torpid animals. Of 27 chipmunks refrigerated, 41 per cent died, 33 per cent remained active, and 26 per cent became dormant. Temperatures below 16° occurred in those which did not recover. This, however, must not be taken to mean that chipmunks cannot survive lower body temperatures, for these experiments were carried on, for convenience, from May to October, with most of them in midsummer, when animals were, presumably, not in the best condition for hibernation. In addition, the change in temperature may have been too sudden. Johnson (1931) states that "sudden severe cold may irritate and finally kill the animal [*Citellus*]."

In a series of eight experiments the blood sugar was lowered by the injection of insulin. It was then an easy matter to induce dormancy at temperatures of 5°–10° C.

TABLE 3

BLOOD SUGAR—SEASONAL CHANGES

Month	Number of Animals Tested	Blood Sugar (In Mg./100 Cc.)		
		High	Low	Average
January	2	112	88	100
February	5	134	103	114
March	4	130	116	122.75
April	5	198	127	159
May	7	191	141	170
June	3	183	169	174.3
July	6	192	169	180.0
August	10	208*	127	163.3
September	4	200	148	167.25
October	17	202	110†	153
November	18	175	74	136.3
December	21	169	61	130.76

* Pregnant. † Immature.

With the lowered body temperature, as would be expected, there was a drop in pulse from a normal average of 250/min. to a range between 76 and 162/min.; respiratory rate likewise decreased from a normal of 100–150/min. to 23–60/min.

Similar observations were made on the thirteen-lined ground squirrel, *C. tridecemlineatus tridecemlineatus* (Mitchell). Two adult male ground squirrels under observation in the laboratory showed consistently very different blood sugar levels while active. When they became dormant following an 8-hour fast and a reduction of the temperature of their surroundings, both showed much lower blood sugar values. Determinations on six different occasions revealed that animal A had an average blood sugar, when active, of 198 mg./100 cc. and of 98 mg./100 cc. when torpid (a loss of 50.5 per cent). The average blood sugar of animal B when active was 152 mg./100 cc. and 88 mg./100 cc. when torpid (loss = 42.1 per cent). It was noted also that when the room temperature was lowered, animal B, the one with lower blood sugar, became torpid at 17° C., whereas animal A was active until the temperature was lowered to 12° C. This represents further evidence that there is a relationship between the blood sugar of a hibernating animal and its activity. It is interesting to note in this connection that the values for these two animals when inactive correspond more closely than when active.

We have found, then, that in chipmunks kept at a temperature of about 20° C. the blood sugar and the weights of the pituitary and adrenal glands are highest in July and lowest in January. When the animals are chilled, even in midsummer, there is a fall in blood sugar similar to, or greater than, that which occurs at room temperature in winter. At the same time a chilled animal, as well as one during normal hibernation, has slower pulse and respiration.

How may these facts be interrelated? One of the ways in which *nonhibernating* mammals react to cold is by increased discharge of adrenalin, as shown so sensationally by Cramer (1928). This has been confirmed by several other workers who have been able to regulate the degree of exhaustion of the adrenal medulla

TABLE 4*

FALL IN BLOOD SUGAR DUE TO LOWERING OF THE SURROUNDING TEMPERATURE

Animal Number	Blood Sugar when Active at Room Temperature (Mg./100 Cc.)	Blood Sugar when Torpid in Icebox (Mg./100 Cc.)	Blood Sugar when Active or Partially Torpid in Icebox (Mg./100 Cc.)	Fall in Blood Sugar Due to Subjection to Cold (Mg./100 Cc.)	Body Temperature when Active at Room Temperature (In °C.)	Body Temperature after Subjection to Cold (In °C.)	Number of Hours in Icebox
9	143	89	.....	54	38	20.5	47.5
18	158	.....	129	29	38.5	.....	50.75
22	141	91	.....	30	37	21.2	46
24	162	97	.....	65	38	22.5	51
25	169	.....	112	57	37.4	24.2	47
28	170	.....	152	18	38.5	32	46
29	167	.....	153	14	37	31	91
31	149	71	.....	78	36	16.8	48
32	151	80	.....	70	37.2	17	22.25
30	127	91	.....	36	37.5	23.7	.....
49	163	99	.....	64	37	29	20
52	158	101	.....	57	37.8	22	20
55	128	97	.....	31	.....	.....	.....
97	112	72	.....	40	36.7	20.1	.....

* All the data in this table are from animals which survived the experiment.

How may these facts be interrelated? One of the ways in which *nonhibernating* mammals react to cold is by increased discharge of adrenalin, as shown so sensationally by Cramer (1928). This has been confirmed by several other workers who have been able to regulate the degree of exhaustion of the adrenal medulla

in cats by the length of time they were exposed to cold (Britton, 1928; Crowden and Pearson, 1928; Crowden, 1929).

In these animals emergency secretion of adrenalin leads to hyperglycemia and increased metabolism, which, in turn, help to maintain body temperature. If their temperature falls, they become more or less torpid. A prolonged exposure to cold may result in enlargement of the adrenal glands, as found by Hartman, Brownell, and Crosby (1931) to occur in rats chilled for 20 or more hours.

While stimulation of the adrenal medulla produces a temporary increase in blood sugar, the maintenance of constant sugar level depends largely upon the adrenal cortex (Britton, 1930; Long, 1938). In chipmunks the adrenal glands are smaller in the fall and winter than in summer. Similar seasonal shrinkage has been found in other *hibernating* mammals, as in spermophiles (Mann, 1916; Zalesky, 1934) and woodchucks (Cushing and Goetsch, 1915).

Zalesky found a proportionately greater decrease in the size of the cortex than of the medulla. Inadequate supply of cortin, then, is probably responsible for the low level of blood sugar found in our chipmunks in winter; by Dubois (1894) and Endres, Taylor, Mathews, and Dale (1930) in the hibernating woodchuck; and by Feinschmidt and Ferdmann (1932) in hibernating *Citellus*. With hypoglycemia goes inability to maintain normal metabolic rate and body temperature.

Since reduction in cortical activity has been found to decrease the proportion of blood plasma (Grollman, 1936), it may also explain the concentration of blood found in winter by Rasmussen (1916) in woodchucks and by Worth (1932) in bats. In Rasmussen's experiment, blood remained concentrated until the animals had had food and water. However, in our experiments, seasonal decrease in adrenal size had no perceptible effect on hemoconcentration.

To summarize:

1. In the eastern chipmunk, *Tamias striatus lysteri*, the erythrocyte count was higher in summer than in winter. However, there was no indication of seasonal change in leucocyte counts.

2. The weights of the adrenal glands increased nearly threefold from a January low to a plateau extending from April through September, then regressed.

3. Blood sugar increased 80 per cent from January to July in animals kept at about 20° C. all year around.

4. In most of the animals tested the blood sugar, even in midsummer, would drop markedly if they were kept 24–48 hours at 4°–6° C.

5. If, as a result of chilling, the blood sugar fell below 100 mg./100 cc., the chipmunks were likely to become torpid.

LITERATURE CITED

Benedict, F. G., and Lee, R. C. 1938. Hibernation and the marmot physiology. Pub. Carnegie Inst. Washington, No. 497.

Britton, S. W. 1928. Adrenin secretion on exposure to cold, together with a possible explanation of hibernation. Amer. Jour. Physiol., **84**:119.

———. 1930. Adrenal insufficiency and related considerations. Physiol. Rev., **10**:617.

Condrin, J. M. 1936. Observations on the seasonal and reproductive activities of the eastern chipmunk. Jour. Mam., **17**:231.

Cramer, W. 1928. Heat regulation, fever, civilization and the thyroid adrenal apparatus. New York: Longmans, Green & Co.

Crowden, G. P. 1929. The replacement of depleted adrenalin in the suprarenals. Jour. Physiol., **68**: 313.

Crowden, G. P., and Pearson, M. G. 1928. The effect of cold on the adrenalin content of the suprarenal glands. Jour. Physiol., **65**:25P.

Cushing, H., and Goetsch, E. 1915. Hibernation and pituitary body. Jour. Exper. Med., **22**:25.

Dubois, R. 1894. Variations du glycogène du foie et du sucre du sang. ... Compt. rend. Soc. biol., **46**:219.

——. 1896. Physiologie comparée de la marmotte. Paris: Masson et Cie.

ENDRES, G.; TAYLOR, H.; MATHEWS, B. H. C.; and DALE, A. 1930. Observations on certain physiological processes of the marmot. Proc. Roy. Soc. London, B, 107:222.

FEINSCHMIDT, O., and FERDMANN, D. 1932. Ueber die chemischen Bestandteile des Blutes winterschlafender Tiere. Biochem. Zeitschr., 248:107.

GROLLMAN, A. 1936. The adrenals. Baltimore: Williams & Wilkins Co.

HARTMAN, F. A.; BROWNELL, K. A.; and CROSBY, A. A. 1931. The relation of cortin to the maintenance of body temperature. Amer. Jour. Physiol., 98:674.

JOHNSON, GEORGE E. 1931. Hibernation in mammals. Quart. Rev. Biol., 6:439.

LONG, C. H. N. 1938. The adrenal cortex and carbohydrate metabolism. Sigma Xi Quart., 26:175.

MANN, F. C. 1916. The ductless glands and hibernation. Amer. Jour. Physiol., 41:173.

POLIMANTI, O. 1912. Il Letargo. Roma: G. Bardi.

RASMUSSEN, A. T. 1916. The corpuscles, hemoglobin content and specific gravity of the blood during hibernation in the woodchuck (*Marmota monax*). Amer. Jour. Physiol., 41:464.

WORTH, R. 1932. Observations on the blood and blood-forming organs of certain local Chiroptera. Folia haemat., 48:337.

ZALESKY, M. 1934. A study of the seasonal changes in the adrenal gland of the thirteen-lined ground squirrel (*C. tridecemlineatus*) with particular reference to its sexual cycle. Anat. Rec., 60:291.

EFFECTS OF X-RAYS UPON FLECKING IN THE DOMESTIC PIGEON[1]

LEON J. COLE
University of Wisconsin

AND

HAROLD E. FINLEY
Morehouse College

INTRODUCTION

THE term "flecking," in relation to plumage pattern in pigeons, is used to designate irregular patches of one color on a background of another. Specifically, the flecks are of a recessive color against a more or less "bleached" dominant ground color. The birds used in this experiment were genetically ash-red and, except for the flecks, phenotypically of that color. The flecks were black or chocolate-colored; these colors are recessive to the ash-red. Hollander and Cole (1940) have suggested that the flecks are due to somatic mutations occurring spontaneously in chromatophore cells within individual feather germs, the flecks being areas composed of the descendants of the mutated cells. If such is the case, it seemed logical that similar mutations might be produced by X-rays, resulting in increased flecking. This was tested in the experiments reported herewith.

Ash-red is sex-linked and is represented by the symbol B^A. Hollander and Cole (1940) pointed out that homozygous ash-red birds (B^AB^A, necessarily males) do not have flecks, and they explained this by the unlikelihood of the mutation occurring simultaneously in both genes of a cell. In males heterozygous for chocolate (B^Ab) the flecks are chocolate, but in males heterozygous for black (B^AB) the flecks are black.

Ash-red females are hemizygous (B^A—) and might be expected to show as much flecking as heterozygous males; but, as a matter of fact, they show relatively little flecking, and such flecks as they have are chocolate. The latter might be antici-

[1] Paper No. 338 from the department of genetics, Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

pated if there is mutation of the B^4 gene, which would allow expression of the residual chocolate color. The decrease in the amount of flecking may not indicate a corresponding decrease in the frequency of the mutations; it may be, rather, that the mutation is of such a nature that the viability of the mutated cells is lessened, so that either their lineage dies out completely or the flecks produced are much decreased in size. This would suggest that the mutation which produces the flecking is of the nature of a partial chromosomal loss or rearrangement. In the male, such a mutation in a cell is compensated by the unmutated chromosome; in the female, there is no compensatory chromosome.

EXPERIMENTAL PROCEDURE

Plucking.—The fact that feathers which are removed start to regenerate at once and are soon replaced gives the opportunity to study a number of feather generations within the time required for a normal molt. Corresponding feathers were plucked from both wings; the right wing was then irradiated, while the left served as a control. Subsequently the feathers were plucked from both wings at the same time, so that the regenerate generations were always comparable. In some experiments plucking was confined to the primary and secondary wing flights; in others a rectangular area, measuring approximately 20 × 25 mm., was denuded, involving the greater and middle wing coverts and some of the lesser coverts. We shall refer to the first pattern of plucking as "limited plucking" and to the second as "extended plucking."

X-ray equipment and radiation.—The radiations were generated by a General Electric, Standard Double Disc, Number G.E., Sp. 140 Unit, operating on 230 volts, 60 cycles. This equipment was generously placed at our disposal through the kindness of Professor M. F. Guyer and Dr. Pearl Claus, of the zoölogy department at the University of Wisconsin.

Only two dosages were employed—namely, 750 r and 1,000 r. Using an oil-bath filter, equivalent to a 1-mm. aluminum filter, and exposing the birds at a target distance of 10 cm., the standardized time of exposure for a dosage of 750 r was 2 minutes, 42 seconds; for a dosage of 1,000 r it was 3 minutes, 6 seconds. Some of the pigeons received a 750-r dosage; others, 1,000-r. In every case the bird was securely fastened in such a way that the right wing was extended but the left wing was folded. Only the right wing was exposed to the X-rays, the rest of the body being completely shielded by lead plates. Exposures were usually made immediately after plucking. Any exception to this procedure will be indicated. Because the plucked area on the left wing was protected from the X-rays, it served as a control area for comparison with the plucked (experimental) area on the right wing.

The color and extent of pigmentation of each pigeon were recorded on special description sheets and by photographs before the experimental treatment. Also, each primary and secondary feather was mounted after plucking, and its origin recorded. Subsequently the mounted feathers were photographed. This procedure made it possible to compare the lengths, colors, and shapes of successive feathers from any particular follicles. It was impracticable to mount the covert feathers plucked from the rectangular areas, but they were saved for future comparisons.

Twenty-four hours after exposure to X-rays each pigeon was examined with a view to determining whether any general ill effects resulted from the treatment. Measuring and recording of the

lengths of flight feathers, in order to compare the growth on the experimental and control wings, was begun 10–12 days after exposure. Subsequently, periodic examinations supplied additional data on the regenerating feathers. The treatment did not cause ill effects, and many of the pigeons used in this investigation are still alive and under continued observations.

Descriptive terminology.—The following abbreviations and definitions will be helpful for brevity and clarity of description.

The primaries are numbered from 1 to 10, beginning with the outermost; the secondaries then begin with 1 and number inward (proximally). The right or left wing is indicated by the corresponding letters, "R." or "L." Thus, the two innermost primaries and the two outermost secondaries of the right wing would be indicated as "Pr. R. 9, 10; Sec. R. 1, 2."

In order to distinguish the feather originally in a particular follicle from those which grew after the first and subsequent pluckings, the successive feather generations are designated as regenerates. Hence: R_0 is a feather present in the follicle before experimentation; R_1 is a feather regenerated as a result of plucking R_0; R_2 is a feather regenerated following the plucking of R_1; etc. As an example, "R_2 Pr. L. 7" would refer to the feather developed, after two pluckings, by the follicle of the seventh primary of the left wing.

No flecking: Feathers devoid of irregular areas of recessive color.

Slight flecking: A condition in which less than one-half the total number of feathers concerned bear flecks; the flecks are sparsely distributed and are less than 0.5 mm. in diameter.

Moderate flecking: A condition in which at least one-half the total number of feathers concerned bear flecks. The flecks may be (*a*) islands of pigment, measuring at least 0.5 mm. in diameter and with one or more such flecks to a feather, or (*b*) many flecks, distributed so as to form a longitudinal pattern on the feather.

Intense flecking: A condition in which each feather concerned bears flecked areas; the areas may cover half or more of the feather.

Pigeon E456A may be taken as an example of the regular procedure. This bird was described on May 29, 1940 (when it was 7 weeks of age), thus: "Red bar; a few small black flecks in primaries and secondaries; tail a fairly even gray." On November 13, 1940, primaries 9 and 10 and secondaries 1 and 2 were plucked from each wing, and the right wing was exposed to an X-ray dosage of 750 r. At the time of plucking, none of these feathers showed flecks. Those which regenerated in the left wing (row R_1 in Pl. I, Fig. 1*A*) were like the R_0 feathers, but the R_1 feathers of the right wing (Fig. 1*B*) were moderately flecked (cf. R_0 and R_1). By successive pluckings without further X-ray treatment, continued regenerate replacements were produced from the same follicles.

RESULTS

Nine of the birds used in these experiments were of the genotype $B^A B$, while one was an ash-red-faded compound ($B^A B^{0f}$). This makes no difference, however, in the present discussion. It will be noted in Table 1 that the regenerated feathers in all ten of the heterozygous birds showed a noticeably increased amount of flecking following the X-ray treatment. In striking contrast to this, no flecking resulted in the three homozygous individuals. One of these, E517K, was homozygous by pedigree; the homozygosity of the other two, $E284L_2$ and $E284P_2$, which were in a segregating fam-

ily, was established by breeding tests. In order to determine whether heavier treatment might produce flecks in these homozygous birds, E284L$_2$ and E284P$_2$ were subjected to a second exposure, similar to the first, 33 days after the first treatment. No flecks resulted, however, from this additional treatment, the regenerates on the treated side continuing like the controls. This indicates either that the higher dosage did not produce mutations with sufficient frequency that two mutations occurred simultaneously in a collar cell or that, if such coincidences did occur, the cells in which they happened did not survive, or at any rate did not give rise to enough tissue to produce visible flecks. The former would seem to be the more plausible explanation.

Persistence of effect.—That the effect produced by the X-ray in the feather-germ tissue is a permanent one is demonstrated by the fact that following the one treatment the resulting flecks continue to reappear in succeeding feather generations (R_1–R_4 in Fig. 1*B*; R_2–R_5 in Fig. 2*B*). They tend, furthermore, to occur in the same areas and to be of the same relative size and of the same general pattern, but in this there is considerable variation. In right primary 9 of E456A (Pr. R. 9, Fig. 1*B*), for example, scattered flecks appear in the inner vane of the first regenerated feather (R_1); in R_2 a heavy, nearly continuous, longi-

TABLE 1

COMPARISON OF FIRST REGENERATE FEATHERS (R_1) OF CONTROL AND TREATED SIDES WITH THOSE FROM THE SAME FOLLICLES BEFORE TREATMENT (R_0)

Pigeon No.	Dosage	Feathers Plucked	Flecking		
			R_0 (Both Wings)	R_1 (Left Wing, Control)	R_1 (Right Wing, Treated)
	Heterozygotes (E257A, B^AB^{of}; all others, B^AB)				
2860W	1,000 r	Pr. 6–10; Sec. 1, 2	Slight on Pr. 6	Same	Moderate
E257A	750	Pr. 9–10; Sec. 1, 2	Slight	Same	Moderate
E284T$_3$	1,000	Pr. 6–10; Sec. 1, 2	Moderate	Same	More on Pr. 8–10, less on others
E456A	750	Pr. 9, 10; Sec. 1, 2	None	Same	Moderate
E456B	1,000	Pr. 3–5	Slight on Pr. 4	Same	Moderate
E456C	1,000	Sec. 1–5	Slight	Same	Somewhat increased
E456E	750	Pr. 6–10; Sec. 1, 2	Slight	Same	Moderate
E456F	1,000	Pr. 6–10	Slight	Same	Moderate
E456G	750	Pr. 6–10	Slight	Same	Moderate; Pr. 9 half black
E456H	1,000	Sec. 1–5	Slight	Same	Somewhat increased
	Homozygotes (B^AB^A)				
E284L$_3$	1,000*	Pr. 4	None	None	None
E284P$_2$	750*	Pr. 1, 3, 5	None	None	None
E517K	1,000	Sec. 1–5	None	None	None

* Exposure repeated 33 days later.

tudinal fleck occupies the same region; in R_3 and R_4 it is still in the same position, but lighter in R_3 and heavier again in R_4. The first regenerate of right primary 10 shows a streak of black along the edge of the outer vane and a small fleck at the tip of the inner vane; R_2 is almost clear but has an inconspicuous black fleck at the extreme tip of the outer vane; R_3 shows a fleck at the tip of the inner vane; and R_4 resembles R_1 in that the outer vane is margined with black, while the pigment in the inner vane is much more extensive than previously. Similar comparisons show about the same degree of resemblance and of divergence in all the feathers. In making these comparisons it should be borne in mind that the dark shading, as distinguishable from the black flecks, in the feathers of pigeon 2860W (Figs. 2*A* and 2*B*) is caused by a suffusion of recessive reddish pigment ("kitiness," in the breeders' terms) and is not concerned in the present discussion. The probable explanation of the variability in pigment pattern in successive feathers from the same follicle has been discussed by Hollander and Cole (1940, p. 22). Uneven relative growth of different derivative cells in the collar is probably the most important factor.

Age as a factor.—Previous observations (Hollander and Cole, 1940) have established that in normal untreated flecked birds the flecking tends to increase in amount and extent with the age of the bird. The same general tendency is evident in the successive regenerations after plucking and X-ray treatment, as may be seen by comparing rows R_3 and R_4 with rows R_1 and R_2 in Figure 1*B* and rows R_4 and R_5 with rows R_2 and R_3 in Figure 2*B*. That this increase must be due to the activity of cells derived from the mutations induced by the X-rays rather than to spontaneous mutations is indicated by the fact that no similar increase has occurred in the feathers from untreated follicles of the left wings (Figs. 1*A* and 2*A*). The only spontaneous flecking observable in the feathers concerned in the left wings of these two birds is a small black spot, roughly 3 mm. long by 1 mm. wide, in the outer vane of the fourth regeneration of primary 9 (Fig. 2*A;* R_5, Pr. L. 9, indicated by arrow [in the photographs small separations in the web of the feather are often difficult to distinguish from flecks]).

The increase in pigmentation in the irradiated wing with successive pluckings is shown even more strikingly in Plate III, Figures 3–7, and Plate IV, Figures 8–11. Figures 3 and 4 are the left and right wings, respectively, of bird E456E before treatment. This bird is a full brother of E456A, previously described. It will be observed that there are a very few small flecks in both wings. The plucking in this case was of the more extended type; that is, it included not only primaries 6–10 and secondaries 1 and 2 but their greater and lesser coverts as well. In Figure 5 the R_0 feathers have been replaced, and the area of plucking is clearly indicated by the increased flecking of the R_1 feathers. The R_2 regenerates (Fig. 7) mark the area in the treated wing even more definitely, while the control wing (Fig. 6) shows no flecks, although there has been the same regeneration of feathers as in the right wing.

The same general relationship holds in Figures 8–11, which are of the same bird (2860W) as Figures 2*A* and 2*B;* this enables comparison, feather by feather. The point of interest here is that the plucking has been continued over a longer period, Figure 11 showing on March 9, 1943, at least the sixth regenerate feathers. At this time the bird was $2\frac{3}{4}$ years of age and had presumably undergone two or three complete molts, in addition to growing the plucked feathers; but the

records of the normal molts are incomplete, and it is possible that these feathers are R_7 or even R_8. However, it will be observed that, in addition to the increased pigmentation in the area of plucking, there is also flecking outside this area, noticeably on the inner secondaries and lesser coverts. At this same time the plucked area of the left wing was still clear of flecks except for small spontaneous flecks on Pr. L. 9. This would seem to indicate that the X-rays had penetrated the overlying feathers to some extent and affected the feather papillae. Such effect would, of course,

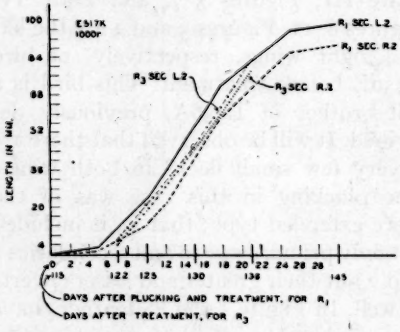


FIG. 12.—Curves of growth of R_1 and R_3 of secondary No. 2 after plucking both wings and irradiation of right wing. Bird E517K.

not be apparent until new feathers were grown after a normal molt. It is also possible that the tissues beginning regeneration in a papilla after a feather has been removed may be in a condition (increased mitosis) more susceptible to the X-rays, although the treatments in these cases followed within 1 hour after plucking.

EMERGENCE AND GROWTH OF FEATHERS

Initial retardation.—A conspicuous effect of irradiation of feather germs is that the regeneration of feathers from such follicles is definitely retarded. The subject of feather growth without and following irradiation has been studied extensively in this laboratory by Mrs. Rose Frisch (1944); so only general observations need be mentioned at this time. Text Figure 12 represents a typical case of feather growth in the left (untreated) and right (irradiated) wings. The feather concerned in this instance is the second secondary of bird E517K, the right wing having been given a dosage of 1,000 r immediately following plucking of the feathers. Five days later the incoming feathers were barely showing (3 mm. and 2 mm., respectively). At 10 days the divergence was considerable; but from that time on, the two growth curves (R_1) are roughly parallel. This general picture holds for all the feathers, whatever their final length; the experimental feather emerges later and lags in growth for a few days, after which both grow at approximately the same rate.

The effect of this initial retardation decreases in later regenerations but is still apparent in R_3 115–45 days after treatment, as is also shown in Figure 12.

As noted, the maximum retardation occurs in the first 10 or 12 days. Measurements were made at this interval after plucking and treatment of 31 primary feathers in the left wings of nine pigeons, and of the corresponding feathers at the same time in the right wings of the same birds. The average length of these feathers in the left wing after this period of growth was approximately 23 mm.; in the right wing, 15 mm. The difference of 7 mm. may be taken as a fair measure of the mean initial retardation in regenerating primaries subjected to 750 r or 1,000 r, the smaller dosage apparently being as effective as the larger.

Delayed treatment.—In one experiment the usual irradiation procedure was changed. Two birds, E456C and E456H, were subjected to the extended pattern of plucking, secondary flights 1–5 and

their coverts being removed. The incoming feathers were allowed to grow undisturbed for 16 days, when the right wings were X-rayed with 1,000 r. The right wing of E456H at time of treatment is shown in Plate V, Figure 13, and the average growth of the five feathers in both wings is charted in text Figure 14.

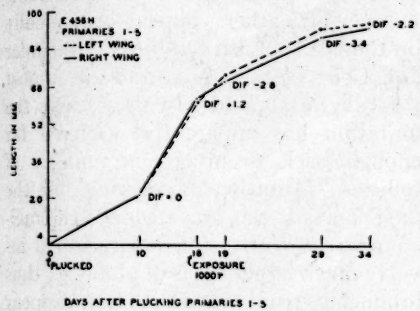


FIG. 14.—Retardation of growth after irradiation delayed until 16 days after feathers were plucked. Curves based on average lengths of secondaries 1–5. Bird E456H.

At 10 days the two sides averaged the same, and there was only a slight and probably insignificant difference at 16 days. It was at this point that the right wing was X-rayed, producing a definite retardation, which was maintained until growth ceased. It is apparent, therefore, that the regenerating feather germ may be affected in such way that growth is retarded even at the sixteenth day, but the degree of retardation seems to be considerably reduced.

Results on the other bird, E456C, were essentially the same; and measurements taken when it was 4 years of age show that the differential effects of the X-ray treatment still persist, though in lesser degree (average difference in 1941, 7.7 mm.; in 1944, 5.5 mm.). All the secondaries measured are sensibly longer in 1944 than when first measured in 1941. The measurements on the two dates are given in Table 2.

It is not to be anticipated that the portions of the feathers grown prior to treatment would show any increase in flecking over the corresponding control feathers of the other wing; and, as may be seen in Figure 13, these were practically free of flecks. The basal parts of those feathers which developed after the treatment, however, as well as later regenerates, showed some increase, though the flecks were not so large nor so extensive as those following treatment immediately after plucking. It would appear that the extent of mutation may be correlated with the degree of retardation caused by the X-rays, but further experimentation will be necessary to determine whether this is regularly the case.

Heavy dosage.—while the data are not included in the present report, we have found that heavier dosages (2,500 r) may produce profound effects on feather growth, abnormal structure, and depigmentation. This is in accord with results reported by De Boer (1940) in the Rhode

TABLE 2

PERSISTENCE OF EFFECTS OF TREATMENT

	February 15 1941	June 7, 1944
Right wing (treated):		
Sec. R. 1.....	95 mm.	100 mm.
Sec. R. 2.....	90	102
Sec. R. 3.....	89	98
Sec. R. 4.....	*12	97
Sec. R. 5.....	88	94
Av.....	90.5 mm.	98.2 mm.
Left wing (control):		
Sec. L. 1.....	100 mm.	104 mm.
Sec. L. 2.....	99	104
Sec. L. 3.....	97	101
Sec. L. 4.....	90	98
Sec. L. 5.....	92	98
Av.....	95.6 mm.	101.0 mm.

* Replacing feather spontaneously dropped.

Island Red fowl with exposures of 2,000–3,000 r. De Boer found that "exposures sufficient to decrease the rate of the first regeneration after treatment had little effect upon subsequent regenerations." The rate of regeneration probably decreases in later regeneration in the pigeon, but the same does not hold for the degree of flecking.

DISCUSSION

The X-ray experiments here reported deal entirely with induced change in the gene B^A. This change appears to be the same in character as the mutations that occur naturally in this gene, but the irradiation sensibly increases their incidence. Spontaneous mutation is much more frequent in the gene for almond, and preliminary trials indicate that if any increase is produced by irradiation it is much less striking than in ash-red. Hollander and Cole (1940) assumed tentatively that almond is allelic to ash-red and designated it B^{Si}. Hollander (1944) has recently suggested that it is in a different allelic series. However, this probably has no significance in the present connection.

In this paper "mutation" is used as a general term, without any commitment as to its nature. Hollander (1944) has recently offered a provisional hypothesis based on chromosomal rearrangement and position effect in the sex chromosome. Further consideration of the nature of the mutations is reserved, however, until a fuller study can be made of the more extensive material at hand, involving both natural and induced changes.

Hollander and Cole (1940) did not localize the site of the mutations further than to suggest that they occur in the collar cells of the feather papilla. Especially in the light of recent work (Watterson, 1942; Rawles, 1944; Willier and Rawles, 1944), it seems safe to conclude that the changes must actually occur in the melanophore cells in the base of the papilla, presumably in the collar. If they were to take place earlier in the melanophore lineage—in the dermis, for example—patches of recessive pigment, involving groups of feathers instead of single feathers, would be more frequent. Spots, including a few to a considerable number of feathers, appear occasionally on the heads of ash-red birds (Hollander and Cole, 1940, Fig. 4) and are almost the rule on almonds. In these cases the mutation has apparently occurred far enough back to involve a number of follicles. Mutations occurring in the melanoblasts, the precursors of the melanophores (Watterson, 1944), would involve much larger areas of plumage, thus producing true mosaics. These appear sporadically from time to time (Hollander and Cole, 1940; Hollander, 1944) but with nothing like the constancy of flecking. Hollander (1944) has classified flecking separately as "variegation," but it would seem that mosaicism and flecking may be due to the same kind of genetic change, differing only in place and frequency of occurrence.

The tendency of flecked areas to be reproduced, but with considerable variation, in successive feathers from the same follicle has been pointed out in this and earlier papers. The amount of variability is greater than would be expected if the mutations occurred in the structural cells of the collar, but is readily explainable by the more or less erratic migration of mutated melanophores.

SUMMARY

1. The occurrence of irregular patches of a recessive color on a background of the dominant color in pigeons heterozygous for the two colors concerned has been termed "flecking" and is attributed to mutation of the dominant gene in cells of the feather germ.

PLATE I

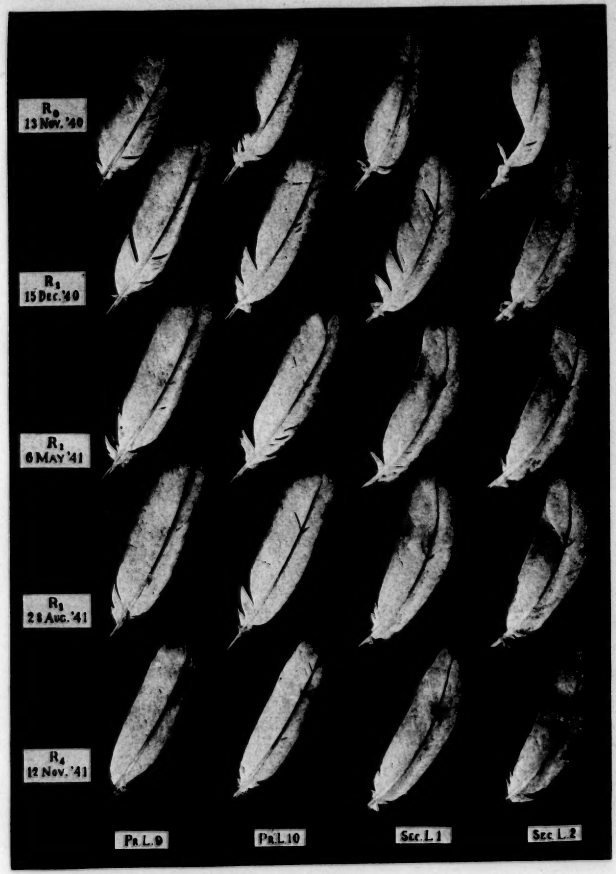


FIG. 1A

FIG. 1B

PLATE II

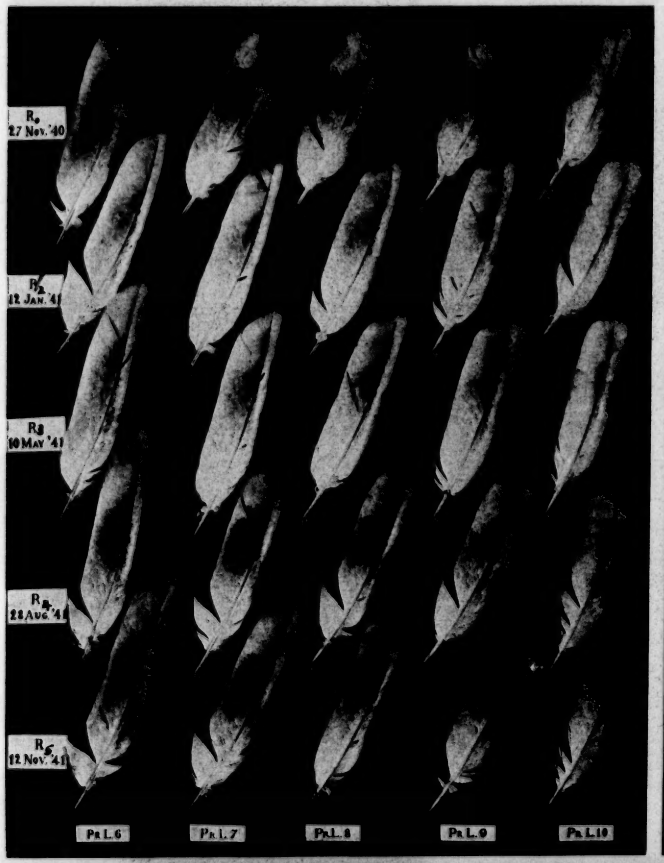
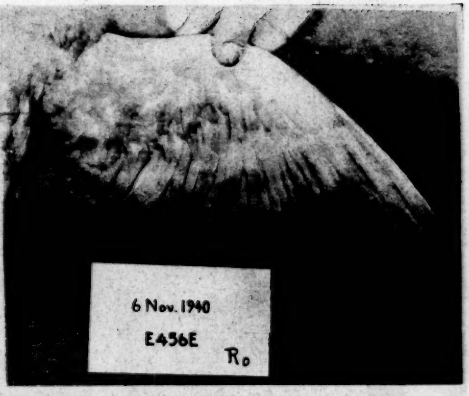


FIG. 2A

FIG. 2B

PLATE III

3



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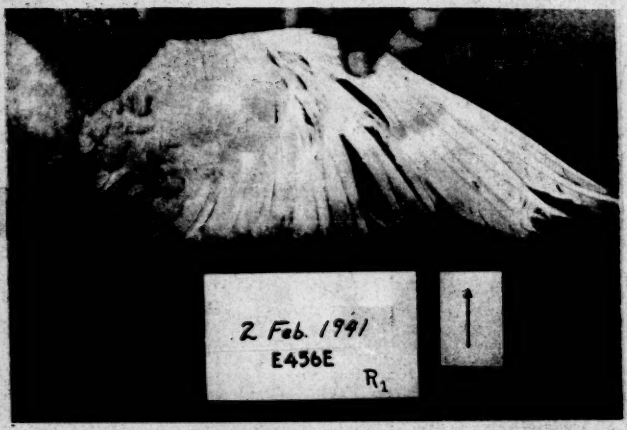

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PLATE IV

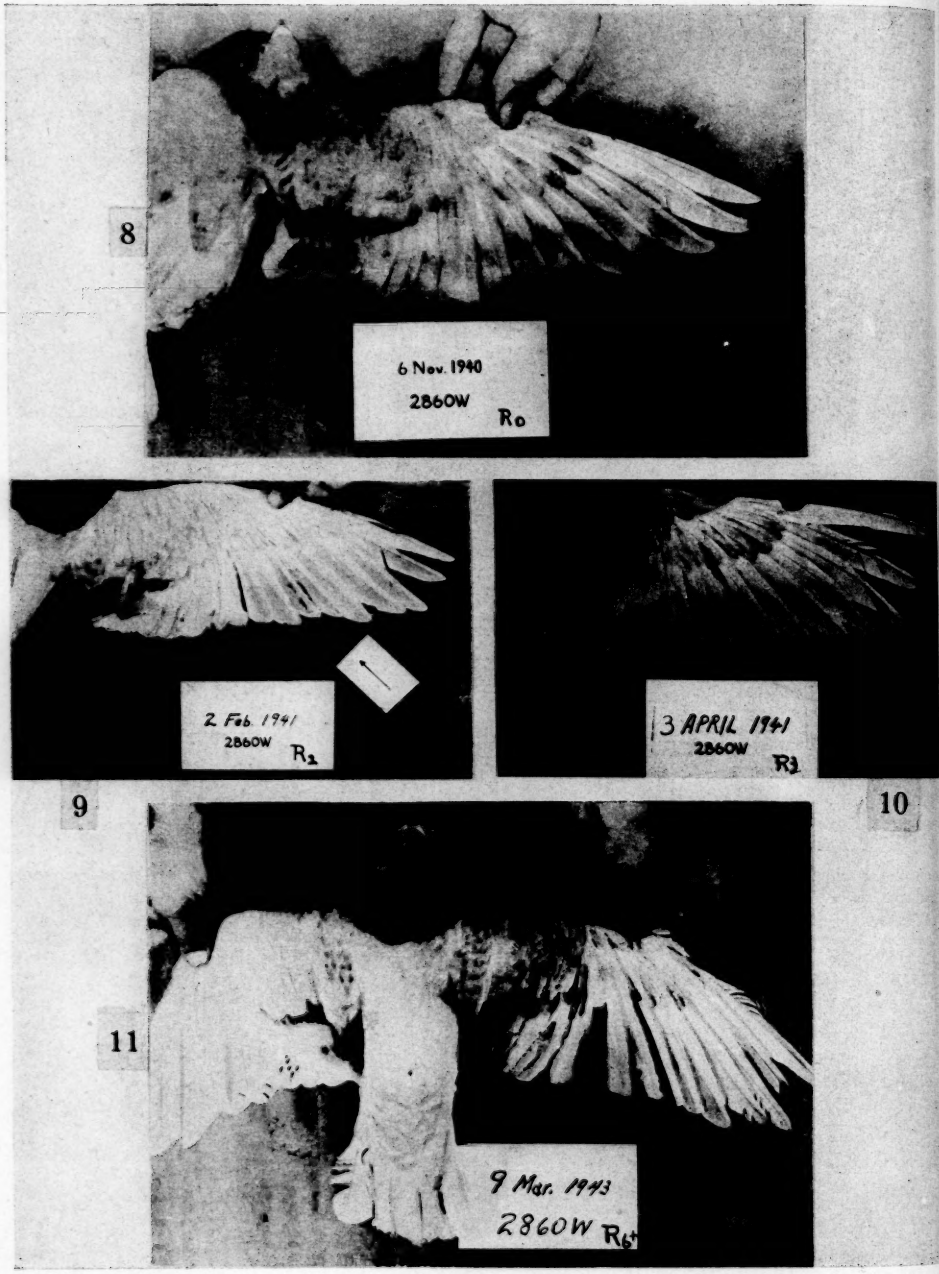
8
6 Nov. 1940
2860W
R0
2 Feb 1941
2860W
R2
3 APRIL 1941
2860W
R3
9
10
11
9 Mar. 1943
2860W

PLATE V

FIG. 13

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2. In such birds the number and extent of the flecks in feathers subsequently regenerated can be definitely increased by exposure of the follicle to X-ray dosages of 750 r and 1,000 r.

3. The increase not only occurs in the first feathers regenerated after treatment but persists with a generally increased intensity in later regenerates from the same follicles.

4. The treatment of a follicle delays the emergence of the new feather, but the growth rate is thereafter practically the same as that of the control.

5. It may be concluded that the X-rays increase the frequency of the same sort of change that occurs spontaneously in the feather germs of pigeons of the genotypes concerned. The nature of this change will be considered more fully at a future time.

LITERATURE CITED

COLE, L. J., and H. E. FINLEY. 1942. The production of somatic mutations in the pigeon with X-rays. Genetics, 27:138 (Abstract).

DE BOER, BENJAMIN. 1940. Effects of X-rays upon regeneration of feathers in the common fowl. Jour. Morph., 67:299–319.

FRISCH, ROSE E. 1944. Growth rates of the primary flight feathers of the pigeon and the effects of X-radiation. Jour. Exper. Zoöl., 97:199–229.

HOLLANDER, W. F. 1944. Mosaic effects in domestic birds. Quart. Rev. Biol., 19:285–307.

HOLLANDER, W. F., and LEON J. COLE. 1940. Somatic mosaics in the domestic pigeon. Genetics, 25:16–40.

RAWLES, MARY E. 1944. The migration of melanoblasts after hatching into pigment-free skin grafts of the common fowl. Physiol. Zoöl., 17:167–183.

WATTERSON, R. L. 1942. The morphogenesis of down feathers with special reference to the developmental history of melanophores. Physiol. Zoöl., 15:234–261.

WILLIER, B. H., and MARY E. RAWLES. 1944. Genotypic control of feather color pattern as demonstrated by the effects of a sex-linked gene upon the melanophores. Genetics, 29:309–330.

PLATE I

FIG. 1*A*.—Left primaries 9 and 10 and secondaries 1 and 2 from pigeon E456A. *Top row*, original feathers plucked; *lower rows*, successive regenerates from the same follicles. No treatment (control).

FIG. 1*B*.—Corresponding feathers from the right wing of E456A. An X-ray dosage of 750 r was given immediately after the R_0 feathers were plucked. Compare the flecking with the control feathers in Fig. 1*A*.

PLATE II

FIG. 2*A*.—Primaries 6–10 from left wing of pigeon 2860W. No treatment. A small "spontaneous" fleck in R_4 of primary 9 (*arrow*). R_1 feathers are not shown.

FIG. 2*B*.—Corresponding feathers from right wing of 2860W. Treatment: 1,000 r on November 27, 1940.

PLATE III

FIGS. 3 AND 4.—Left and right wings, respectively, of pigeon E456E on November 6, before plucking and treatment (of right wing) with 750 r. Very small amount of flecking in both wings.

FIG. 5.—Right wing of E456E with R_1 feathers, replacing those originally plucked, showing a decided increase in flecking.

FIGS. 6 AND 7.—Left and right wings of E456E with R_2 feathers in plucked areas. In the right wing they show a further increase of flecking, but there is no increase in the left wing.

PLATE IV

FIGS. 8–10.—Right wing of 2860W. Individual feathers may be compared with those in Figs. 2*A* and 2*B*. Fig. 8: before treatment, R_0 feathers; Figs. 9 and 10: primaries 6–10 and their coverts replaced by R_2 and R_3 feathers, respectively, following treatment. Note gradual increase in flecking.

FIG. 11.—Pigeon 2860W, showing R_6 or later feathers in both wings. Not only has the flecking increased still further in primaries 6–10 and their coverts in the right wing, but it has extended somewhat to other areas. The left wing is still unflecked (apparent flecks are artifacts).

PLATE V

FIG. 13.—Right wing of pigeon E456H at time of treatment with 1,000 r, the treatment being delayed for 16 days after secondaries 1–5 and their coverts were removed. Average length of the new secondaries was 62.2 mm.

SOME ASPECTS OF THE DOMINANCE-SUBORDINATION RELATIONSHIP IN THE FISH PLATYPOECILUS MACULATUS[1]

JAMES CONGER BRADDOCK
Whitman Laboratory, University of Chicago[2]

THIS paper is not offered as a complete analysis of the social behavior of *Platypoecilus maculatus* but rather as an introduction to the topic. Descriptions of the actual bodily movements of individual fishes approach the "natural-history" type of presentation. This approach is open to criticism, but it does permit the reader more clearly to visualize the characteristic behavior of the fishes; the movements of individuals of this species are stereotyped enough and the experimental procedures are sufficiently precise to permit this type of treatment. The work on this problem to date has been exploratory, and the hypotheses presented are tentative. Further experimental analyses are planned.

Dominance-subordination relationships have been demonstrated for many vertebrate types (Collias, 1944). The pioneer work in this field was done by Schjelderup-Ebbe, who in 1922 described in detail the social order in chickens and referred to various social relationships in other birds in a more general way. Allee and his associates have reported a series of detailed studies on the subject, and Allee (1931, 1938, 1940, 1942, 1943) has worked toward integrating many of the social factors into an orderly plan. The late G. K. Noble and his associates (1936, 1938, 1939*a*, *b*, 1940, 1941) did the preliminary work on social orders in fishes, with special attention to the cyclid *Hemichromis bimaculatus* (Noble and Curtis, 1939) and the poecilid *Xiphophorus helleri* (Noble, 1939; Noble and Borne, 1940).

THE EXPERIMENTAL ANIMAL AND ITS TREATMENT

Platypoecilus maculatus was chosen as the experimental animal for this work because of its conveniently small size, relatively high belligerency, and color patterns that are variable enough to make identification of individual fishes certain even when they are in continual and fairly rapid motion. The fishes were obtained in part from local dealers, in part from large-scale breeders.

The various experimental conditions are presented in tabular form in Table 1. The lamps referred to in the table are ordinary goosenecked student lamps. The artificial pond water[3] is a preparation which experience has shown to be a favorable medium for the maintenance of various fresh-water animals. All of the aquaria contained gravel but no plants, with the exception of the large greenhouse tank, which had plants at both ends but not in the middle portion.

An effort was made to begin taking observations at the same time each day. Also, the various aquaria were always observed in the same order.

[1] The information presented here is the result of studies made at the University of Chicago. I wish to express warm thanks to Dr. W. C. Allee for his guidance in the prosecution of this work. I wish also to thank Mr. Asher J. Finkel for his help with the statistical analysis.

[2] Present address: University of Idaho, Moscow, Idaho.

[3] Formula for artificial pond water: distilled H_2O plus the following salts: 100 mg/liter, $CaCl_2 \cdot 4H_2O$; 50 mg/liter, K_2SO_4; 50 mg/liter, $MgSO_4 \cdot 7H_2O$; 50 mg/liter, $NaNO_3$.

Experiment I was the original experiment and was designed simply to find whether homosexual small groups have a social hierarchy.

Experiment II employed the fishes used in Experiment I, but they were assembled according to rank—i.e., all of the top-ranking fishes from the various aquaria in Experiment I were placed together in one aquarium for continued study. The same was done for the various other ranks. The sexes were not mixed. The experiment was designed to find whether the top-ranking fishes were innately more aggressive or less social than those occupying lower positions in a hierarchy and whether they would establish rank orders as readily as did unselected groups.

Experiment III was designed to allow comparison between heterosexual and homosexual groupings. New fishes that had not been used in previous experiments were procured for the purpose.

At the beginning, Experiment IV duplicated Experiment I. However, as soon as hierarchies had become established, the most dominant individual in each aquarium was interchanged with the most dominant individual in another aquarium. Observations were made at the time of the transfer and for 30 minutes thereafter. Males and females were not mixed in this process. The exchange was made twice during the course of the experiment, and general observations were continued for almost 3 weeks. Experiment IV tested the reactions of an established hierarchy toward a strange fish and the reactions of the strange fish to the new group.

Experiment V concerned territoriality. By using a fairly large tank containing plants and open water, it was hoped that a sufficient variety of conditions was present to allow the fishes to establish territories if such was their normal tendency.

TABLE I

MAKEUP OF THE VARIOUS EXPERIMENTS*

Experimental Conditions	Expt. I	Expt. II	Expt. III	Expt. IV	Expt. V
Location	W.L.	W.L.	W.L.	W.L.	W.L.G.
Type of water	a.p.	a.p.	a.p.	a.p.	tap
Temperature of water	75° F.†	75° F.†	75° F.†	75° F.†	variable
Lighting	L.F.	L.F.	L.F.	L.F.	daylight
No. of aquaria	8	8	6	8	1
Size of aquaria	30.5×26.5×26.5 cm.				8′×2′×1′3″
No. of fishes in aquarium	4	4	4	4	10
Origin of fishes	new	Expt. I	new	new	new
No. of aquaria containing ♀♀ only	4	4	0	4	0
No. of aquaria containing ♂♂ only	4	4	0	4	0
No. of aquaria containing equal numbers of ♂♂ and ♀♀	0	0	6	0	1
Duration in days	33	26	26	18	20
No. of days of observations made	20	15	20	16	10
Length of observation periods (in minutes)	30	30	30	30	60

* W.L., Whitman Laboratory; W.L.G., Whitman Laboratory Greenhouse; a.p., artificial pond water; tap, tap water; L.F., Fluorescent and ordinary electric lights.

† Approximate temperature.

DEFINITIONS

The term *nip* is used to designate the characteristic contact reaction between

two fishes. Essentially this is a quick bite, involving no noticeable harm to the fish that is bitten. Because *Platypoecilus* has a dorsally placed mouth, the nips tend to be directed upward and are received on the ventral part of the body. When possible, the nipped fish retreats upward in the most direct path of escape. A *nip* is analogous to a *peck* in the so-called "peck-order" studies with birds.

The term *challenge* refers to a characteristic type of behavior wherein a belligerent fish erects its dorsal fin, spreads its caudal fin, and fans the water with its pectoral fins. At the same time it bends its body into a shallow S-shape. The head is higher in the water than the tail.

The *submission* behavior in this species involves lowering of the dorsal fin, collapsing of the caudal fin, and a general drooping of the entire body. A submission reaction also involves backing slowly away for 2 or 3 inches. The animal then turns and flees.

Nip right is a form of dominance behavior in which one fish may nip another fish without being nipped in return.

Nip-dominance behavior, which does not occur in *Platypoecilus*, would involve steady reciprocal nipping but with one member of a contact-pair winning the majority of nip contacts.

A *contact-pair* is composed of any two fishes that have established, or are in the process of establishing, a dominance-subordination relationship.

FORMATION OF INITIAL HIERARCHIES

GROUPS OF FOUR FEMALES

When four *Platypoecilus* females are first brought together, each sinks to the bottom and indicates her alertness by erecting her dorsal and spreading her caudal fin. Each fish remains in this state for a few seconds or even several minutes.

Soon one individual becomes less alert, as indicated by the return of the dorsal and caudal fins to their usual positions. She begins to cruise slowly about until she encounters another fish. When the distance between the two fishes is approximately 2 inches, both exhibit challenging behavior. In this posture the two drift slowly until one turns suddenly upon the other and nips her. If nipping occurs, it will continue at intervals until the victim shows submission. Actually, the sequence seldom is completed at the first meeting. Indeed, the first encounter usually involves mutual challenging alone. In other instances an attack follows, but there is no noticeable submission by the victim. Under such circumstances the erstwhile aggressor may become the victim at the next meeting. A lasting dominance-subordination relationship is not visibly established until the victim submits to the aggressor without challenging by either fish.

While the first pair is reacting as a temporary contact-pair, the other two fishes usually exhibit similar behavior. Their series of meetings appears to be precisely similar to that of the first pair.

During the intervals between her aggressive meetings with a particular fish, an individual usually does not avoid the other fishes except when they are engaged in challenge reactions. Any encounters between lone fishes will result in challenging. As a result, all possible dominance-subordination relationships are usually established within a short time (about 30 minutes was the shortest time observed). An exception occurs when one fish is especially aggressive. Such an individual interferes actively with the contact-pair reactions of the others by attacking the challenger of a third fish. This aggressiveness results in the establishment of the nip order between the overly active individual and

the other three fishes before all of the other dominance-subordination relationships are settled. Under such circumstances, as many as 7 days may be required for the working-out of the complete social order.

GROUPS OF FOUR MALES

When a group of four *Platypoecilus* males is first assembled, the behavior of the individuals composing the group resembles that of females under the same conditions. The same social tension exists. There is the same mutual challenging and the same establishment of contact-pairs. However, certain differences are apparent almost immediately. The males tend to remain longer in one spot; their movements are more jerky and rapid; and their nipping appears to be more intense. Because the males tend to move only to challenge or to fight, while the females move about the aquarium almost continually, males meet in contact-pairs less frequently than do females, and more time is required for their social order to be worked out. The least time observed for the formation of a complete set of relationships among four males was 1 day; the greatest, 12 days.

Male dominance-subordination relationships tend to be established one at a time rather than more or less simultaneously, as with females. Thus, each of the first two contact-pairs in a newly established group of males may continue with an extended series of contact reactions until their dominance-subordination relationship is fully determined. Usually, the two original contact-pairs do not break up, nor are new contact-pairs formed until this has happened.

One of the two original contact-pairs frequently establishes its dominance subordination relationship before the other. Its members then separate, and one or both may interfere with the other initial pair by challenging or attacking either of its members. Such behavior may disrupt this pair, for one of them may react to the new challenge instead of the original one. Under these conditions the two original contact-pairs may not be the first to establish their relationships.

HETEROSEXUAL GROUPS

Taken as a whole, the general behavior of a group of *Platypoecilus* consisting of two males and two females has much in common with that of homosexual groups of four fishes. The initial period is much like that of a homosexual female group in that the fishes are rather steadily on the move. The males swim about much more than in male homosexual groups; but they move in a jerky manner, in contrast to the smoother swimming of the females. No breeding behavior was noted during the formative period in any of the six aquaria observed in Experiment III. The establishment of dominance-subordination relationships tends to follow the male, rather than the female, pattern; and the males tend to occupy the more dominant positions in the group, even at the beginning of its social history. This was true in five of the six aquaria involved in the experiment.

HIERARCHY TYPES AND THEIR DISTRIBUTION

The establishment of a set of dominance-subordination relationships for contact-pairs automatically results in the establishment of a hierarchy. Frequently this will be a *true straight-line* hierarchy, in which each individual dominates all others whose rank is lower than its own. This may be considered the completion of the social integration of the group. Such hierarchies were observed many times.

It is not uncommon to observe a set of

nip relationships in which the contact-pairs are not all formed or are of such a nature that it is impossible to recognize a complete hierarchy. Overtly, these incomplete hierarchies are of two types: a *pseudo-straight-line* hierarchy, one in which no fish is out of line but each fish does not nip all individuals whose rank is lower than its own, and an *incomplete hierarchy proper*, one in which the position of one or more individuals is undetermined. Apparently, the incomplete types do not differ in kind from the true straight-line hierarchy, but they occur frequently enough to justify naming them. These are illustrated in Table 2 by different stages in the evolution of the final social order in aquarium No. 1 of Experiment I.

TABLE 2

TYPES OF HIERARCHIES

Type of Hierarchy	No. of Fishes Dominated	Individuals Dominated
I. True straight-line:		
B	3	D.C.A.
D	2	C.A.
C	1	A.
A	0	
II. Pseudo-straight-line:		
B	3	A.C.D.
D	1	C.
C	1	A.
A	0	
III. Incomplete proper:		
B	3	A.C.D.
C	2	A. D.
A	0	
D	0	
IV. Non-straight-line:		
D	2	A.C.
A	2	C.B.
B	1	D.
C	0	

Not all hierarchies are of the straight-line type. In some, one or more individuals are out of line. This is analogous to the triangle situation as seen in chickens. I have chosen the term *non-straight-line hierarchy* to describe a social grouping of this type. The example given in Table 2 is from aquarium No. 4 of Experiment II.

The true straight-line hierarchy occurs when there is not too great a difference between the belligerency of the individuals. The incomplete types occur most frequently when one individual, usually the most dominant fish, attacks the other fishes so steadily that they are unable to work out all their interrelationships. It is also possible that in some cases the relationships have been worked out, but the dominant fish does not permit the less aggressive individuals to exercise them. The non-straight-line hierarchy can be explained at this time only on the postulate that the fishes recognize each other as individuals, as do hens (Schjelderup-Ebbe, 1922).

The distribution of the various hierarchy types seems to differ in the different sexual arrangements. Differences of equal magnitude may be noted between aquaria containing groups of similar sexual composition. The figures presented in Table 3 are totals which may show a trend in distribution and are offered for what they may be worth. It may well be that factors other than sex are the major determiners of the type of hierarchy. On the basis of Table 3, true straight-line hierarchies are most likely to occur in homosexual female groups and least likely to occur in heterosexual groups. Pseudo-straight-line hierarchies have about the same frequency in each of the three groupings. Incomplete hierarchies proper are most common in heterosexual groups and least common in homosexual male groups. Non-straight-line types occur most frequently in male

homosexual groups, least frequently in female homosexual groups.

THE EXISTENCE OF NIP RIGHT

The dominance relationship upon which the hierarchies are based is of the nip-right type; i.e., any despot has the right to nip its subordinates but is not normally nipped by them. This phenomenon is not quite so apparent here as is peck right in the case of chickens. The slight obscurity results from the instability of the hierarchies. Reverse nipping occurs but constitutes a very small percentage of the total amount of nipping done (Table 4). Also, reverse nipping is intermittent, not continuous, and thus represents either accidental nipping resulting perhaps from lack of recognition of a more dominant fish or else an attempted revolt against a despot. Until further data are available, it is not clear what significance underlies the observed differences in reverse nipping in the different combinations.

TABLE 3

DISTRIBUTION OF HIERARCHY TYPES (FOUR FISHES)

Type of Hierarchy	Females (Expt. I)		Males (Expt. II)		Heterosexual Groups (Expt. III)	
	No. of Daily Hierarchies	Percentage	No. of Daily Hierarchies	Percentage	No. of Daily Hierarchies	Percentage
True straight-line...	43	53.8	32	40.0	23	18.3
Pseudo-straight-line.	11	13.8	12	15.0	17	13.5
Incomplete proper..	20	25.0	16	20.0	72	57.2
Non-straight-line...	6	7.5	20	25.0	14	11.1

BEHAVIOR IN ESTABLISHED HIERARCHIES

GROUPS OF FOUR FEMALES

The behavior of *Platypoecilus* females after a hierarchy has become established differs somewhat from that shown earlier. There is a lack of the social tension so characteristic of individuals that are strange to each other. Under the influence of an established society the fishes move about freely. There is little challenging, and such as does occur indicates attempted revolts by subordinates. Most challenging evokes an immediate attack by the challenged despot, with the result that there is little mutual challenging. Nipping in an established hierarchy appears to be less intense than in the formative period except when a despot is thwarting an attempted revolt. Any despot may interfere with the contact-pair relationships of her subordinates and break them up by nipping either of the fishes involved.

The females are not uniformly active but alternate between relatively long periods of activity and relatively shorter ones of inactivity. General inactivity is initiated when any one of the four individuals enters a secluded portion of the aquarium and remains there. Soon a second fish will settle down in another secluded spot or corner or will come to rest upon the bottom of the aquarium. This process continues until all four fishes have become quiet. During the inactive period that ensues the individual fishes may move about, one at a time, but their movements are slow, and they avoid the vicinity of others.

An inactive period ends when any one of the four fishes begins to move about the aquarium rapidly and nip her subordinates when she encounters them. The nipped animals swim on actively and may nip their own subordinates. Thus in a few seconds the four fishes may proceed from the inactive to an extremely active phase of their social behavior.

GROUPS OF FOUR MALES

After a hierarchy has become established, the behavior of *Platypoecilus* males does not change so much as it does in a group of females. Social tensions persist; the same jerky movements and intense nipping may be observed. However, challenging—especially mutual challenging—is infrequent. As before, males tend to move about less frequently and with less smoothness of motion than the females exhibit at a comparable stage in group life.

TABLE 4

DISTRIBUTION OF REVERSE NIPPING
EXPERIMENTS I AND III

Grouping	Percentage of Reverse Nipping	Total No. of Nips
Homosexual:		
Females nip females	0.8	13,059
Males nip males	2.4	9,355
Heterosexual:		
Females nip females	1.4	1,984
Males nip males	0.3	3,495
Nips between males and females	0.8	5,766

The alternating periods of inactivity and activity noted for the females have their counterpart in the male groupings, but the inactive periods of the males are proportionately longer. Social activity is characterized by a series of events such as occurs with the females, but the male activity is more intense throughout.

During periods of inactivity, even when the males are not engaged in contact-pair reactions, their postures indicate alertness. In this they differ from the females, which seem to be completely at rest during their period of social quiet.

Male nipping is generally more vigorous than female nipping, but males do less nipping than the females. In Experiment I the females averaged 81.6 nips, while the males averaged only 58.5 nips, per fish per hour.

HETEROSEXUAL GROUPS OF TWO MALES AND TWO FEMALES

The behavior of the fishes in an established heterosexual group resembles that shown by a homosexual group of females. Fishes of both sexes move about the aquarium rather steadily during any active period. The relatively greater activity of the males, as compared with that in male homosexual groups, is notable and may well be related to the presence of the females, which move about in their normal manner and thus keep the males in motion. The stimulus furnished by the females is social rather than primarily sexual, for the females keep the males in motion even during periods when no breeding activity can be noted. The jerkiness of movement of the males is not quite so noticeable as it is under homosexual circumstances. Periods of inactivity occur here, as elsewhere; they are relatively less frequent than in groups of males and no more frequent than in groups of females. Challenging is infrequent and commonly indicates an attempted revolt.

Males nip individuals of both sexes, and females do the same (Table 5). Males and females divide their nipping rather equally between the sexes except during breeding periods, when males mainly nip other males. During such periods a male will follow a sexually receptive female and attempt to mate with

her. If the female challenges, as she often will, the male may nip her, but rarely otherwise. In half of the aquaria in Experiment III the females gave the majority of their nips to other females; in the other half, males received more of the nips given by their associated females.

TABLE 5

HETEROSEXUAL GROUPS, EXPERIMENT III
DISTRIBUTION OF NIPPING
ACCORDING TO SEX

Sexual Combinations	Total No. of Nips	Percentage
Male nips male	3,495	31.1
Male nips female	3,327	29.6
Female nips female	1,983	17.6
Female nips male	2,439	21.7

In heterosexual groups males nip more than do females—approximately 54.1 nips per fish per hour as contrasted with 35 nips for females. This is in sharp contrast to the situation in male and female homosexual groups, where the females do more nipping. Examination indicates that we are here dealing with a decrease in the female nipping rate rather than an increase in that of the males. No explanation for this fact is offered at this time except the possibility that the sometimes severe nipping of the males causes the females to spend more time escaping from them and less time in aggressive behavior toward other fishes. The greater intensity of male nipping gives them a social advantage in heterosexual groups. This is expressed as a strong tendency for males to be despots in such groups (Table 6).

SOME ELEMENTS OF SOCIAL BEHAVIOR IN ESTABLISHED GROUPS

Within the framework of a true straight-line hierarchy there is a steady downward progression in the amount of nipping done by fishes occupying the several ranks. This is generally true for homosexual groups of both sexes and for heterosexual groups as well (Table 7). Thus the amount of nipping done by any individual corresponds to the position of that individual in the hierarchy.

This was true for each of the four aquaria containing females in Experiment I and for three of the four aquaria containing males. In the fourth aquarium the score was 142 nips for the No. 1 rank, against 163 for the No. 2. In the heterosexual groupings of Experiment III the amount of nipping corresponded to rank in five of the six aquaria used, while in the sixth the No. 1 and No. 2 positions were precisely equal, with 65 nips apiece. In both aquaria where the differences were out of line with the general trend the amount of nipping was at a minimum.

A despot does not distribute its nipping evenly among its subordinates, nor does it always nip its subordinates according to rank. Rather, the despot nips each individual subordinate differentially (Tables 8, 9, and 10).

TABLE 6

HETEROSEXUAL GROUPS, EXPERIMENT III: DISTRIBUTION OF NO. 1 RANK IN TRUE STRAIGHT-LINE HIERARCHIES

Aquarium No.	Days Males Held Top Rank	Days Females Held Top Rank
1	21	0
2	18	3
3	12	2
4	1	7
5	4	8
6	5	6
Totals	60 (69.8%)	26 (30.2%)

The rank of the fish most likely to receive the majority of the nips given by a superior fish varies from aquarium to aquarium, regardless of the type of sexual grouping used (Table 8) and regard-

less of whether the nips of the alpha or beta fish are used in the analysis (Table 9).

Marked differential nipping is received by different individuals (Table 10). The evidence given in Tables 8 and 9 indicates that this is not directly related to differences in rank; it may result—at least in part—from the recognition of, and reaction to, individuals as such.

The stability of any hierarchy is a function of the stabilities of the various subrelationships within the hierarchy, such as the length of time one fish can dominate one, two, or three others. As would be expected, considering the instability of the hierarchies, such subrelationships are usually short-lived; in fact, the greater number of cases observed endured only for 1 day (Table 11). It should be noted that instability may not be a fundamental characteristic of *Platypoecilus* societies at all times and under all conditions. Further work may show that the size of the aquarium is a factor in stability. Also, long-term observations may provide evidence of an increasing stability as time passes. Reference to Table 11 will show that the relationships of certain individuals were of exceptionally long duration. There were 11 cases among the females of Experiment I in which one female dominated another for 29 days—the entire observation period for that experiment. Similarly, in the same experiment there were 5 cases wherein one female succeeded in dominating two others for 29 days. While these cases and similar ones among the males (Expt. I) and the heterosexual grouping (Expt. III) may be regarded as exceptional under the conditions of these experiments, they constitute evidence

TABLE 7

AMOUNT OF NIPPING DONE BY THE FISHES OCCUPYING EACH OF THE FOUR POSITIONS IN TRUE STRAIGHT-LINE HIERARCHIES

Sex	Expt.	No. 1 Fish		No. 2 Fish		No. 3 Fish		No. 4 Fish	
		Nips	Per Cent	Nips	Per Cent	Nips	Per Cent	Nips	Per Cent
Females	I	4,886	63.5	2,292	28.9	481	6.3	31	0.4
Males	I	2,214	59.7	1,168	31.5	309	8.3	16	0.4
Heterosexual	III	1,505	59.7	808	32.0	202	8.0	5	0.2

TABLE 8

TRUE STRAIGHT-LINE HIERARCHIES: DISTRIBUTION OF NIPPING BY THE NO. 1 FISH

Aquarium No.	No. 2 Fish	No. 3 Fish	No. 4 Fish
	Females, Experiment I		
1	655	379	383
2	474	555	377
3	492	246	443
4	249	422	342
	Males, Experiment I		
5	41	163	31
6	129	7	6
7	219	317	306
8	274	627	94
	Heterosexual, Experiment III		
1	159	117	107
2	30	9	26
3	7	4	18
4	103	24	87
5	36	114	167
6	356	103	53

that stability is possible. The factors making for stability are, at present, unknown.

The differences between the mean durations of each of the subrelationships (Table 11) were tested for statistical significance; none was found. Unless the lack of statistical significance can be attributed to the small number of cases tested or to an unrepresentative sample, it appears that the more complicated relationships are as likely to endure as the less complicated ones; i.e., one fish can dominate two or three others as easily as one. This would be evidence for individual recognition.

Wholly similar comparative studies were made of the durations of the subrelationships between homosexual male, homosexual female, and heterosexual groupings; this was done statistically by testing the mean differences for significance. None was discovered for the more complicated relationships, possibly because of the small number of cases involved. However, in all cases the male homosexual and heterosexual durations were shorter than those of the homosexual female grouping—significantly so for one male dominating one of two individuals. No significant differences were noted between the male homosexual and the heterosexual relationships.[4]

Changes in rank occur frequently among these fishes within any hierarchy, and the frequency of such changes is a measure of the instability of the relationships. Such changes result from successful revolts and are characterized by a period or a succession of periods of mutual challenging lasting from a few seconds to as long as 30 minutes. Mutual challenging appears to be an outward sign of a period of temporary equality between the members of the contact-pair involved, since a truly dominant fish will attack its subordinate without challenging it. Most of the observed revolts involved the reversal of only one contact-pair relationship. However, a fish that has overthrown one of its despots may continue its course of self-emancipation until it has overthrown a second or even a third despot. This disruptive behavior may create such chaos in the aquarium that contact relationships between other individuals will be reversed.[5] The foregoing account holds

TABLE 9

TRUE STRAIGHT-LINE HIERARCHIES: DISTRIBUTION OF NIPPING BY THE NO. 2 FISH

Aquarium No.	No. 3 Fish	No. 4 Fish
	Females, Experiment I	
1	1,277	533
2	520	281
3	630	159
4	22	100
	Males, Experiment I	
5	117	17
6	42	120
7	352	387
8	54	71
	Heterosexual, Experiment III	
1	40	151
2	60	5
3	26	1
4	61	5
5	30	130
6	112	79

[4] Detailed data have been recorded elsewhere (Braddock, 1942) in a form similar to that shown in Table 11, and only the respective means are given here.

[5] Aquarium No. 2, Expt. I, ♀ ♀, March 2–3. The hierarchy changed from D–B–C–A to A–B–D–C. Thus, contact pairs AB, AD, and BD were reversed.

Aquarium No. 3, Expt. I, ♀ ♀, February 17–20. The hierarchy changed from D–C–B–A to A–D–C–B. Thus, AB, AC, and AD were reversed.

good for all of the sexual groupings observed. However, revolts are most likely to affect more than one contact-pair in the case of homosexual female groups (44 per cent) and least likely to do so in homosexual male groups (28 per cent). Heterosexual groups resemble the female homosexual groupings in this respect (42 per cent).

The frequency of successful revolts in each of the sexual groupings may be used as an index to the relative stabilities of the groupings. On this basis the female homosexual groups are the most stable. The male homosexual and heterosexual groupings are approximately equal to each other in stability, but both are less stable than the females.[6] Successful revolts constitute a better index of relative stability than reverse nipping, since the latter may occur by accident or in connection with unsuccessful revolts. At present there is insufficient evidence to determine which, if any, of the several subordinate ranks is most likely to overthrow a despot.

TABLE 10

DIFFERENTIAL NIPPING ACCORDING TO INDIVIDUAL FISHES

Aquarium No.	A–B	A–C	A–D	B–A	B–C	B–D	C–A	C–B	C–D	D–A	D–B	D–C
	Females, Experiment I											
1	0	0	10	789	434	1,293	496	510	178	17	0	57
2	66	13	38	294	362	152	166	0	0	581	613	1,134
3	1	16	152	181	3	0	229	991	1	468	291	642
4	27	117	0	1	132	0	0	0	0	778	866	960
	Males, Experiment I											
5	201	4	17	89	703	36	148	1	154	40	46	523
6	298	25	25	24	0	388	276	1,254	3	39	10	588
7	59	0	0	45	0	26	683	201	90	328	640	214
8	0	90	159	466	1,064	120	6	0	213	0	50	25
	Heterosexual, Experiment III											
1	5	0	1	4	1	0	634	481	476	152	390	0
2	9	5	1	47	8	13	482	18	0	267	95	412
3	450	17	4	0	5	0	0	7	0	40	131	468
4	677	563	30	58	157	58	5	44	828	203	8	0
5	2	130	32	431	754	19	8	0	7	58	14	312
6	299	531	76	2	10	24	17	123	713	34	116	159

HOMOSEXUAL GROUPS SELECTED ACCORDING TO THEIR SEVERAL RANKS IN EXPERIMENT I

Experiment II was designed to test the importance of general aggressiveness in hierarchy formation and maintenance. In each aquarium in Experiment I one individual held the No. 1 position for a greater number of days than any other fish. The same was true for each of the other three positions. On this basis it was

[6] Relative numbers of successful revolts: ♀♀, homosexual groups, 0.061 revolts per fish per day; ♂♂, homosexual groups, 0.118 revolts per fish per day; and heterosexual groups, 0.132 revolts per fish per day.

possible, at the conclusion of Experiment I, to designate each fish as a No. 1, 2, 3, or 4 individual.

In Experiment II all of the No. 1 females were assembled in one aquarium. All of the No. 2 females were placed in another aquarium. The same was done for the females of the other ranks and for the males. Thus, Experiment II exactly duplicated Experiment I with the single exception that an attempt was made to place together individuals of a similar degree of aggressiveness.

The results were not a duplication of those of Experiment I. True straight-line hierarchies were rarer both for males and females selected for rank, and incomplete hierarchies were more common (Table 12). The amount of reverse nipping increased from 0.8 per cent for females selected at random to 1.8 per cent for females selected for rank. The males showed a similar increase (2.4–4.3 per cent). These data are given with some reserve; yet the indicated trend may be real.

TABLE 11

EXPERIMENT I: DISTRIBUTION OF DURATION OF VARIOUS DOMINANCE RELATIONSHIPS FOR FEMALES, TOGETHER WITH MEANS FOR SIMILAR DATA FOR MALES AND FOR BISEXUAL GROUPS

Duration in Days	One Fish Dominates One Other	One Fish Dominates Two Others	One Fish Dominates Three Others	Three Fishes Maintain Same Hierarchy	Four Fishes Maintain Same Hierarchy
No. of cases	63	47	13	79	35
Duration in days					
1	38.1%	36.2%	30.8%	48.1%	62.9%
2	4.7	2.1	.....	8.9	17.1
3	3.2	6.4	7.7	3.8	5.7
4	3.2	4.3	7.7	6.3	5.7
5	1.6	.....	.....	3.8	2.9
6	6.3	4.3	.....	8.9	2.9
7	.....	.....	.....	2.5	2.9
8	6.3	8.5	15.4	7.6	.....
9	.....	4.3	7.7	1.3	.....
10	.....	.....	.....	1.3	.....
11	6.3	12.8	15.4	3.8	.....
12	.....	.....	.....	1.3	.....
13	1.6	.....	.....	.....	.....
14	1.6	2.1	.....	1.3	.....
15	1.6	2.1	.....	.....	.....
16	1.6	4.3	7.7	.....	.....
18	.....	.....	7.7	.....	.....
22	.....	.....	.....	1.3	.....
23	4.7	2.1	.....	.....	.....
26	1.6	.....	.....	.....	.....
29	17.5	10.6	.....	.....	.....
Totals	99.9	100.1	100.1	100.2	100.1
Mean, females	9.9 days	8.4 days	7.1 days	3.8 days	1.9 days
Mean, males	5.8	4.3	3.2	2.9	1.5
Mean, bisexual	5.0	4.4	3.9	2.3	1.2

The total amount of nipping underwent a decrease from 81.6 to 47.9 nips per fish per hour for females and from 58.5 to 24.4 nips per fish per hour for males selected for rank.

As was to be expected, the several subrelationships tended to be short-lived, the greatest number of examples lasting only 1 day (Table 13). In the case of the females the mean duration of

the time one fish dominated one other or one fish dominated two others was significantly shorter for fishes selected for rank. The males showed no significant differences for any of the relationships.

The above information tends to indicate that general aggressiveness is important and that groups composed of individuals with a similar degree of this quality have more difficulty in forming and maintaining hierarchies than do groups selected at random. It seems logical that any two fishes that are almost equally aggressive will have more difficulty in establishing and maintaining a stable dominance-subordination relationship than two with much variation in aggressiveness. This is reflected throughout the whole hierarchy. It is not suggested that general aggressiveness is the only factor in dominance behavior among these fishes, but in the early weeks of a given group relationship it is more important than the rank held at the moment.

TRANSFERS TO ESTABLISHED HIERARCHIES

Experiment IV was designed to study the reactions of a fish newly introduced into an established hierarchy and the reactions of the original members of the hierarchy to the stranger. Otherwise the experimental setup situation duplicated that of Experiment I—i.e., homosexual groupings of four fishes with four aquaria for each sex. After the original groups had been together long enough to make possible the ranking of the individuals by the method used in Experiment II—

TABLE 12

DISTRIBUTION OF HIERARCHY TYPES: MALES AND FEMALES, EXPERIMENT II

Hierarchy Types	Females		Males	
	No. of Daily Hierarchies	Percentage	No. of Daily Hierarchies	Percentage
True straight-line	15	27.8	7	13.0
Pseudo-straight-line	9	16.7	14	25.9
Incomplete proper	25	46.3	23	42.6
Non-straight-line	5	9.3	10	18.5

TABLE 13

DISTRIBUTION OF DURATIONS OF VARIOUS DOMINANCE RELATIONSHIPS MALES AND FEMALES, EXPERIMENT II

Dominance Relation	Females		Males	
	No. of Cases	Mean	No. of Cases	Mean
One fish dominates one fish	66	6.3	79	4.5
One fish dominates two fishes	47	4.9	59	2.5
One fish dominates three fishes	12	4.3	12	1.7
Three fishes maintain same hierarchy	61	2.5	51	2.2
Four fishes maintain same hierarchy	22	1.5	27	1.1

i.e., by customary rank rather than rank at the moment—the fish that was usually No. 1 was removed from each aquarium and placed in another; all were No. 1 or No. 2 fishes on the basis of rank at the moment of transfer. The number of individuals in each aquarium remained the same, but there was a stranger in each group. The sexes were not mixed in making the transfers. Observations were made for the 30-minute period immediately following the exchange or for a longer time, if necessary, and for the regular half-hour periods on succeeding days. After these new groupings had established their hierarchies, a similar rearrangement was made, and a second set of observations was recorded.

Under the above conditions an individual newly introduced into an established hierarchy invariably exhibited submissive reactions to any fish approaching it. The resident fishes attacked the stranger almost immediately. In all but 2 cases the highest-ranking fish attacked first, followed by the others—not necessarily in the order of their rank. No challenging was observed—an indication that the fishes native to the aquarium recognized their advantage over the new fish. The behavior during this initial period was essentially the same for male and female groups.

Shortly after the initial period of submission the foreign individual began to challenge the members of the established order. For males the minimum time that elapsed before this happened was 5 minutes; with females the minimum time was more than 30 minutes. The maxima were 28 minutes for males and 2 days for females. From then on the new fish established its relationships with the other fishes one by one and finally achieved a position in the hierarchy above the omega one occupied when first introduced.

TERRITORIES

Territoriality has been reported for birds in connection with peck-order studies in the case of ring doves (Bennett, 1939), geese (Jenkins, 1944), canaries (Shoemaker, 1939), and others and for some of the nest-guarding fishes (Breder, 1936; Noble and Curtis, 1939). Noble and Curtis (1939) have reported the existence of breeding territories in the cyclid *Hemichromis bimaculatus*. The phenomenon has not been observed in any of the live-bearing fishes. The association between territoriality and *peck-dominance* rather than *peck-right* organization has been summarized by Allee (1942).

The large tank employed in Experiment V had plants at both ends and a clear space in the middle. It was believed that enough variety of environment existed to permit the fishes to find suitable territories if it were their nature to do so. Only ten individuals—five males and five females—were used, in order that the fishes could avoid each other and thus have an opportunity to take over territories without undue interference.

No evidence of territorial behavior was noted in Experiment V. The fishes tended to school into a loosely integrated group; and, so far as could be observed, the contact-pair relationships were the same on a given day anywhere in the aquarium. There has been no evidence of territoriality in any of the other observations.

THE PROBLEM OF INDIVIDUAL RECOGNITION

There is little exact evidence in this work that bears on the problem of the possible individual recognition of one fish by another. There are two lines of available evidence. One of these lies in the frequency of differential nipping of sub-

ordinate fishes by the common despot, regardless of rank in the hierarchy. Differential nipping may indicate individual recognition, or it may result from differential reaction to differing behavior patterns without actual recognition of associated fishes as individuals. Thus, any fish that gives a reaction approaching a "challenge" is nipped by its despot, and the No. 3 fish may give such reactions more than the individual in the No. 2 position. The despot may then react to this tendency, not to the individual as such.

A second line of evidence comes from the existence of non-straight-line hierarchies. Such arrangements are marked by a high-ranking individual being regularly nipped by a fish that occupies a generally low position in the social order. In other words, nipping triangles or even quadrangles may exist in place of a straight-line order. If the outcome of pair contacts depended on chance alone, 25 per cent of all hierarchies in groups of three fishes should be of the non-straight-line type.[7] Actually, about 9 per cent of 640 hierarchies were of this sort.

When four fish are considered, if all pair contacts were won as a result of chance, 62.5 per cent of the hierarchies would be non-straight-line. Actually, about 16 per cent of 160 observed hierarchies were of this kind. The strong indications are that the outcome of these pair contacts is not a matter of chance; and, although non-straight-line hierarchies suggest individual recognition, it seems likely that this is only one of a number of factors acting in the establishment and maintenance of social ranks in *Platypoecilus*. Finally, the series of events following the introduction of a strange fish into an established hierarchy may give some evidence of individual recognition. While the foreign fish may well be reacting to the strangeness of its new environment and to the threatening behavior of a whole set of fishes, rather than to its new associates as strange individuals, there seems to be little doubt that the resident fishes are reacting to the introduced fish as an individual whose strangeness is detected.

[7] The number of possible straight-line hierarchies is indicated by factorial $n(n!)$, where n is the number of individuals present. The total number of different kinds of ranked arrangements of individuals is $2[n(n-1)/2]$. I am indebted to Professor Sewall Wright for these formulas.

FACTORS INVOLVED IN DOMINANCE

It is not a simple matter to account for dominance in specific terms. There is evidence that dominance is the result of intricately interwoven qualities, some of which are at present unknown. Furthermore, in fishes the relative importance of the various known factors has not been determined.

Perhaps general aggressiveness itself is the most important single factor known to help determine the dominance potential of an individual; yet aggressiveness is a complex as yet unanalyzed for any animal. It seems probable that future work will break this composite down into more basic factors and discover the role played in aggressiveness by such factors as size, sex, and certain psychological considerations, together with others as yet unknown. At present the only objective measure known for aggressiveness in these fishes is that furnished by nipping. Certainly there exists a close correspondence between an individual's capacity for dominating others and the amount of nipping done by that individual over a period of time. It has been shown (Table 7) that a more dominant fish will do more nipping than a less dominant fish. Furthermore, the data on amount of nipping, and on the number of days each fish dominated each other fish in Experiments I, II, and III were tested

for correlation. The r-values are given in Table 14, and they indicate a fairly close correlation. If there were no interrelationship between amount of nipping and dominance, such a close correlation would be most unlikely. For instance, A might be despot over B for 10 days and nip B 500 times during that period; after a reversal that resulted in B becoming despot over A, B might then dominate A for 5 days and nip A 1,000 times over that period. Such examples have not been observed. The foregoing evidence indicates that a close interrelationship exists between dominance and the amount of nipping done.

TABLE 14

CORRELATION BETWEEN HIGH RATE OF NIPPING AND DOMINANCE

Expt.	Sex	r
I	Females	0.894
I	Males	0.893
II	Females	0.74
II	Males	0.82
III	Heterosexual	0.73

The next question is whether nipping is a privilege conferred by dominance and having no significance yet noted or whether it is an instrument of social control. By "social control" is meant the maintenance of hierarchy stability through stability of the several contact-pair relationships. While the answer to this question is not as yet entirely clear, there are indications that the latter alternative is the more probable. The homosexual female groupings combine the highest nipping rate with the highest measure of hierarchy stability observed in these experiments. The homosexual male groupings exhibit less nipping and less stability. Thus, it is suggested that constant nipping by a despot may tend to discourage revolts and thus maintain the dominant position of the despot. If this is true, we can conclude that aggressiveness produces nipping, which is one factor in the initiation and maintenance of dominance.[8]

Another factor is size (Table 15). The available data present no exact, over-all correlation between size and dominance because the actual size of each fish was not measured. (The less one handles these fishes, the healthier they remain.) However, the various pair combinations were analyzed to find what percentage of the time the fish judged to be larger dominated a smaller one. There are indications that, under random conditions, size is a more important factor for males than for females. This is true whether the males are in homosexual groupings with other males (Expt. I) or in heterosexual groupings with females (Expt. III). In the case of contact relationships between males and females, size is less important than in unisexual lots of males and somewhat more important than in unisexual female groups. When the fishes were grouped according to rank previously obtained (Expt. II),

TABLE 15

FREQUENCY WITH WHICH THE LARGER OF TWO FISHES DOMINATED THE SMALLER FISH

Expt.	Sexual Grouping	Percentage of Larger Fish Dominated
I	Females, homosexual	50.6
	Males, homosexual	69.7
II	Females, homosexual	72.6
	Males, homosexual	58.8
III	Heterosexual, all cases	62.2
	Females and females	57.6
	Males and males	71.0
	Males and females	58.0

[8] The other possibility, i.e., that fishes nip because they are dominant, must not be neglected. If this is correct, the role of nipping in social life is undetermined.

indications were that the importance of size as a dominance factor was increased for the females and decreased for the males. Actually, size was now a more important factor for the females than for the males. The increase in the importance of size for females selected for rank seems to be a result of the process of selection which tended to equalize factors other than size but not size itself, since size is unimportant for females under random conditions. Thus, females selected for rank could still have much variation for size, and it could be emphasized beyond its usual importance. Males selected by the same process would tend to be more similar in size, and inequalities in other factors would receive the emphasis.

That maleness is a factor in dominance is demonstrated by the fact that 69.8 per cent of the No. 1 fishes in true straight-line hierarchies of four fishes (two males and two females) were males. This should be considered in connection with the fact that nipping is at its lowest level as a dominance factor under heterosexual conditions and that size is also relatively unimportant.

Psychological factors also enter the picture. The fact that a foreign fish submits without challenging when placed with fishes whose hierarchy is established brings this out. The nature of these psychological factors remains to be discovered.

There is no doubt but that there are other undiscovered qualities in the dominance potential; for there are occasional cases on record where a female fish dominated a male fish that was larger than herself and did more nipping. It is probable also that differential nipping, as applied to individual recognition and even aggressiveness, should be included among the psychological factors. In summary, the dominance potential is a result of aggressiveness; objectively considered, it is related to nipping, size, maleness, and psychological factors, together with many unknowns. A fish does not have to exceed another in all of the values that together constitute its dominance potential in order to occupy high social rank. It may be comparatively deficient in one or more of the measurable factors and still dominate another, probably because it has the advantage in the more crucial attributes that make for dominance.

DISCUSSION

In a general way the social behavior of *Platypoecilus* resembles that of chickens, as reported by Schjelderup-Ebbe (1922), Allee (1943), and others. Nip right appears to be analogous to peck right and is established in the same manner, i.e., by fighting or by passive submission. True straight-line hierarchies are characteristic of chickens, and the triangle situation is one form of non-straight-line hierarchy. There is much evidence that chickens maintain their relationships upon a basis of individual recognition; and the same appears to hold, at least in part, for the fishes studied here. Such differences as have been noted are quantitative. Among the fishes, non-straight-line hierarchies occur more frequently, and triangles and incomplete hierarchies are more characteristic. However, there is a strong tendency toward the straight-line relationship. This is apparent when one considers that non-straight-line relationships do not appear as frequently as they would if chance alone determined the outcome of all contact reactions. When the special conditions under which these observations were made are considered, the stability of *Platypoecilus* societies is definitely less than that of similar societies of hens. Groups of seven or eight hens may maintain the same rela-

tive positions for several months, while a day or two is the usual limit for *Platypoecilus*. However, it should be noted that a few of the contact relationships observed lasted 29 days; this indicates that such relationships may have stability under special circumstances.

Noble (1939) reported what is here called *nip right* in the poecilid fish, *Xiphophorus helleri;* and his descriptions would fit, in a general way, the nip behavior of *Platypoecilus*. This is to be expected, since the two interbreed freely and are similar in their general habits. Differences between the behavior of *Xiphophorus* as reported by Noble and that of *Platypoecilus* are largely differences in the stability of the hierarchies. Noble states that the nip order of a "small school" of *Xiphophorus* may remain constant for 2 or 3 weeks. While individual contact-pair relationships among *Platypoecilus* fishes have been known to last as long as 29 days, this was by no means characteristic of them and is still less so for hierarchies of four fishes. It seems possible that Noble's observations were made under different conditions from those reported here. It is not clear whether Noble began his observations immediately after the fishes were placed together or waited for some time before beginning observations. Thus, Noble's results may represent the situation present after the fishes have been together for a considerable time. Until observations designed to clarify this point have undergone analysis, this must remain an open question. Noble also reports a much stronger tendency toward the true straight-line hierarchy than was noted for *Platypoecilus*. This may also result from differences in the conditions under which the two sets of observations were taken.

The problem of the value of dominance-based societies to their own members and to the species at large is far from solved, but it has been ably discussed by other investigators (Allee, 1943; Collias, 1944; Noble, 1939). Noble adopted the point of view that dominance behavior is primarily concerned with natural selection of individuals. Allee (1938) and Collias (1944) also recognize the possibility of individual selection and, in addition, advance the hypothesis that hierarchies based on individual-against-individual competition may lead to the selection of the whole organized group in competition with other groups. It seems likely that both types of selection are important but under slightly different conditions.

That the survival potential of the whole group may be increased by dominance behavior is suggested by the following lines of evidence. Allee and Guhl (1942) have reported that hens in organized groups gain more weight, eat more, and lay more eggs than those in unorganized flocks. Taken in connection with the fact that fishes are most vulnerable to predators when very young, evidence that very young *Xiphophorus* have nip orders (Noble, 1939) seems significant. *Xiphophorus* adults do not protect their young in any way. Cyclid fishes protect their young; and, when thus protected, the young fishes are not organized into hierarchies (Noble and Curtis, 1939). However, if young cyclids are separated from their parents, they immediately develop social orders (Noble and Curtis, 1939), perhaps as a substitute for parental care. Adult cyclids, pugnacious fighters, do not exhibit dominance behavior (Noble and Curtis, 1939). Male *Bettas* also have no dominance-based societies (Noble, 1939); they, too, are noted for their pugnacity. Female *Bettas* are less pugnacious than males and have social orders (Noble, 1939). Male *Platypoecilus* are more pugnacious

than females and have less stable social orders. While it is entirely possible that the evidence for fishes merely indicates that the more pugnacious types have less ability to form hierarchies, it is also possible that social groups are an alternative to ability for self-protection. As such they would protect the group as a whole rather than any particular individual in the group.

Evidence also exists that a dominant position within a group is correlated with a greater individual survival potential (Allee, 1938; Collias, 1944). Noble (1939), working with fish, reported that the more dominant individuals in a group of *Xiphophorus* lose less weight during periods of starvation than do more subordinate individuals. Noble also found that the larger and heavier fishes tend to occupy the positions of greater dominance. Evidence in the present paper indicates that a similar situation exists for *Platypoecilus*.

Both individual and group selection are important, since, if a species is to survive, it must have both quality and numbers. Within a social unit, selection of individuals will act to maintain quality, while selection of entire groups will maintain sufficient numbers.

SUMMARY

1. Small homosexual or heterosexual groups of *Platypoecilus* fishes exhibit nip-right behavior.

2. The fishes form hierarchies based upon individual contact-pair relationships which are established by challenging and fighting.

3. The hierarchies show a tendency toward the true straight-line type, but incomplete hierarchies and triangle situations are common.

4. After initial hierarchies are established, the social behavior of the fishes is less savage than during the formative period.

5. Under the conditions of these observations all contact relationships tended to be short-lived and were characterized by frequent reversals, due to revolts.

6. Established societies are characterized by alternating periods of activity and inactivity.

7. Despots of either sex will interfere with the contact reactions of their subordinates.

8. A despot of either sex nips its subordinates differentially as individuals and not according to their several ranks.

9. Males are less social than females. They nip less but more savagely, move about less, and their hierarchies are less stable.

10. In heterosexual groups males and females nip individuals of both sexes except during breeding periods, when males tend to limit their nipping to other males.

11. In heterosexual groups the differences between male and female behavior are similar to those in homosexual groups but are less marked.

12. Males tend to occupy the higher ranks in heterosexual hierarchies.

13. The amount of nipping done by a fish corresponds to its rank.

14. A strange fish reacts submissively to all members of an established society. They, in turn, react aggressively toward the stranger.

15. No evidence of territorial behavior was noted.

16. Some evidence exists that the fishes recognize each other as individuals.

17. Factors involved in dominance are: general aggressiveness and other psychological factors, size, maleness, and factors as yet unknown.

LITERATURE CITED

ALLEE, W. C. 1931. Animal aggregations: a study in general sociology. Chicago: University of Chicago Press.

———. 1938. The social life of animals. New York: W. W. Norton.

———. 1940. Concerning the origin of sociality in animals. Scientia, **34**:154–60.

———. 1942. Social dominance and subordination among vertebrates. A chapter in Levels of integration in biological and social systems, ed. R. REDFIELD. Lancaster, Pa.: Jacques Cattell Press.

———. 1943. Where angels fear to tread: a contribution from general sociology to human ethics. Science, **97**:517–25.

ALLEE, W. C., and GUHL, A. 1942. Concerning possible survival value of socially organized as compared with disorganized groups of hens. Anat. Rec., Suppl., **84**:497–98.

BENNETT, M. A. 1939. The social hierarchy in ring doves. Ecology, **20**:337–57.

BRADDOCK, J. C. 1942. Some aspects of the dominance-subordination relationship in *Platypoecilus maculatus*. Ph.D. thesis on file in University of Chicago Library.

BREDER, C. M., JR. 1936. The reproductive habits of the North American sunfishes (family Centrarchidae). Zoologica, **21**:1–48.

COLLIAS, N. E. 1944. Aggressive behavior among vertebrate animals. Physiol. Zoöl., **17**:83–123.

GUHL, A. M., and ALLEE, W. C. 1944. Some measurable effects of social organization in flocks of hens. Physiol. Zoöl., **17**:320–47.

JENKINS, D. 1944. Territory as a result of despotism and social organization in geese. Auk, **61**:30–47.

NOBLE, G. K. 1936. The function of the corpus striatum in the social behavior of fishes. Anat. Rec., Suppl. 3, **64**:64 (abstract).

———. 1938. Sexual selection among fishes. Biol. Rev., **13**:133–58.

———. 1939. The experimental animal from the naturalist's point of view. Amer. Nat., **73**:113–26.

NOBLE, G. K., and BORNE, R. 1940. The effect of sex hormones on the social hierarchy of *Xiphophorus helleri*. Anat. Rec., Suppl., **78**:147 (abstract).

———. 1941. The effect of forebrain lesions on the sexual and fighting behavior of *Betta splendens* and other fishes. *Ibid.*, Suppl., **79**:49 (abstract).

NOBLE, G. K., and CURTIS, B. 1939. The social behavior of the jewel fish, *Hemichromis bimaculatus*. Bull. Amer. Mus. Nat. Hist., **76**:1–76.

SCHJELDERUP-EBBE, T. 1922. Beitrage zur Sozialpsychologie des Haushuhns. Zeitschr. f. Psychol., **88**:225–52.

———. 1935. Social behavior of birds. Chap. XX in Murchison's Handbook of social psychology, pp. 947–73. Worcester, Mass.: Clark University Press.

SHOEMAKER, H. H. 1939. Social hierarchy in flocks of the canary. Auk, **56**:381–406.

SOME EFFECTS OF THIAMIN ON THE WINNING OF SOCIAL CONTACTS IN MICE[1]

ELIZABETH A. BEEMAN AND W. C. ALLEE
Whitman Laboratory, University of Chicago

MALE mice establish social hierarchies when caged together. The social order is based on the results of active fighting or on passive submission to a threatening mouse or even to one that does not exhibit any aggression that was apparent to the observer. Aggressive behavior among mice brought together for staged encounters may include one or more of the following activities directed toward another mouse: tail-rattling, hair-fluffing, mincing (see p. 199), threats, chasing, attacks, and fighting. It is possible that these grosser aspects of aggressive behavior would not often be exhibited in groups of mice that have been continually caged together.

A given combat between two mice may last only a fraction of a second or may continue for several minutes. It may consist of a dart or a snap; or there may be extended periods of active, rough-and-tumble fighting that sometimes continues

[1] We are indebted to Dr. J. P. Scott and to Dr. Benson Ginsburg for their critical reading of this paper in manuscript.

until one mouse is killed. These facts have been established in part by Uhrich (1938), in an extensive study of groups of five albino mice caged together, and by Ginsburg and Allee (1942), who experimented upon the fighting behavior of mice brought together in pairs in staged encounters.

Uhrich observed simultaneously the activities of groups of mice in several adjacent cages. Fighting was sufficiently infrequent so that he could usually watch all combats closely without missing any fights. Fighting did not necessarily occur in each of Uhrich's cages during each day of observation. In fact, no fighting was observed on over one-fifth of the 4,931 cage-days on which his report is based. The days without fighting were normally interspersed among others in which active combats were seen in the given cage. There were, however, some groups of mice that apparently did not fight for several days on end. Most frequently, Uhrich found that a group of five mice was organized with only two social ranks—one occupied by a dominant mouse and the other by four equally submissive mice. Other more or less transitional orders were fairly common.

J. P. Scott (1942) discovered in 1940 that several inbred strains of mice developed at the Jackson Memorial Laboratory show prefighting differences in aggressiveness. Ginsburg and Allee (1942), using the work of Uhrich and of Scott as a basis, found that individual mice may be conditioned, with care, to become more aggressive as a result of repeated successes and that others may fairly readily lose aggressiveness following a steady series of defeats. Mice tend to establish the habit of winning or of losing. In part as a result of such conditioning, the social order in communally penned mice, or the fighting success (or lack of it) in mice kept in isolation between staged encounters, tends to be most stable in the upper and lower extremes.

As will be described in some detail in the following section, our mice were housed separately after weaning and were brought together periodically for staged pair-contacts. Mice that are not constantly caged together cannot form a true social organization, such as Uhrich (1938) described for his animals. As with much of the work reported by Ginsburg and Allee (1942), the ranks recorded in the present study are based entirely on the outcome of staged pair-contacts in a neutral cage. Whenever "social order" is mentioned in describing our experiments, we mean the ranking determined by a series of such pair-reactions (see p. 200, next section).

The order so determined is more readily compared directly with the rank-order of tennis players or of pugilists than with the organized social gradient in a small flock of hens or a small herd of cattle. But Ginsburg and Allee (1942) tested the relation between the ranking developed in such a series with the social order shown later in the same mice when caged together and found substantial similarity.

The present series of experiments extended from October, 1941, to May, 1944. They were directed toward continuing the analysis of the factors that make for success in fights and other social contacts of male mice. Because of current interest in the role of thiamin in various biological processes and because of our need for information about the relative importance of diet, as compared with conditioning, we undertook to compare the effect of a surplus or a deficiency of thiamin in the diets of mice on their aggressiveness and their ability to win in staged encounters.

Mice have both strong and weak points as experimental animals for such

a study. On the one hand, they are good experimental subjects—among other reasons, because of the rapidity with which thiamin avitaminosis sets in. On the other hand, they are considerably less than ideal since, unlike hens and many other animals, social dominance may shift readily, often with no apparent cause, or, equally unpredictably, a given social order may remain stable for several weeks or even months. For our purposes it was helpful that the outcome of pair-contacts is most consistent for mice in the alpha or the omega position; shifts in the intermediate ranks occurred more frequently.

ANIMALS AND THEIR TREATMENT

The mice used in this study were descendants of stocks of C57 black mice that originally were given to us by the Jackson Memorial Laboratory at Bar Harbor (see Ginsburg and Allee, 1942). The stocks had been bred through at least fifteen generations of brother-sister matings before they reached us; such inbreeding was continued in the majority of cases, though some line-breeding was necessary to continue our stock. Since the C57 stock has been inbred for so many generations, all animals in our colony should be fairly homogeneous with respect to heredity but not necessarily as regards behavior, because the latter depends in part on experience.

Only adult male mice—littermates or from litters born a few days apart—were used in these experiments. The mice were isolated as soon after they had reached 21 days of age (time of weaning) as possible, depending on when cage space was available. The mice were usually isolated between 22 and 59 days of age, with a mean of 34 days. In a highly exceptional case, No. 36*a* was 120 days old when isolated. All mice used in a single experiment were isolated at approximately the same time. The ears of each mouse were clipped in a pattern that indicated its number, and tail pigment pattern was recorded for additional identification.

All the mice were kept in a well-ventilated, dark room, the temperature of which normally was held at 23° C. during the winter months and at 25° during the summer. Fluctuations between limits of 20°–30° occurred very infrequently, with no observed effect upon the combativeness of the mice. Ceiling lights were turned on for varying periods of time during the day.

Mice were bred and isolated in screen and sheet-metal cages that measured $7 \times 7\frac{1}{4} \times 12$ inches. A few days before staged encounters were to begin, each mouse was transferred from the small cage with a sheet-metal bottom in which he had been isolated to a coarse-meshed-wire cage, $9.5 \times 9.5 \times 7$ inches in size. Except during staged encounters the mice remained isolated in these larger cages throughout the experiment. All cages were scrubbed in hot water once each week; fresh wood shavings were placed in the sheet-metal cages and in trays under the wire-mesh cages. The excreta fell through the mesh floors of the larger cages onto the wood shavings.

According to Williams and Spies (1939), feces may include unabsorbed residues of thiamin originating in the food, and synthesis of thiamin may occur in the intestinal tract. The body is not capable of storing large supplies of vitamin B_1 against a time of future need, but tends to waste what it cannot immediately use. Since mice may eat their own feces, it was important to use cages with wire-mesh floors so that they could not have access to any thiamin which might be present in their feces.

EXPERIMENTAL PROCEDURE

As the study progressed, the experimental techniques were modified; variations will be explained where necessary, but in general the procedure was as follows:

Opportunities for one mouse to meet and to fight another were given according to the pattern established by Ginsburg and Allee (1942). Groups of mice met in staged encounters in round-robin fashion, so that each mouse met every other mouse in the experimental group. The order of encounters was rotated, so that mice did not meet other mice in the same sequence each time.

Encounters between two mice were held in neutral wire cages (mesh, $\frac{5}{16}$ inch) with sheet-metal bottoms; these measured 9.5 × 9.5 × 12 inches and were divided into two equal sections by a galvanized iron partition that could be readily raised. The transfer of a mouse between the home cage and the neutral, or so-called "fighting cage," was done as follows: A piece of wire mesh was placed horizontally in the open door of the home cage and was kept there until the mouse climbed on it of its own accord. Mouse and wire were transferred gently to the open door of the neutral cage, where the mouse eventually climbed off. Although this method of transfer was often time-consuming, since a mouse sometimes would take 10 or 15 minutes before climbing on the wire mesh, it was considered to be a more uniform procedure, as far as handling the mice was concerned, than transferring the mice in the box in which they were routinely weighed, since some mice would enter the box immediately while others had to be chased around the cage before being caught in the box. During an entire experimental period the mice actually were touched by the experimenter only when they were lifted by the tail to be placed in a clean cage; they were never handled just before or after being in the fighting cage.

During an encounter all ceiling lights were turned off and a 60-watt lamp was placed about 7 inches above the fighting cage. This procedure kept the mice from reacting to movements outside the cage. Encounters were started by raising the partition between the two mice. Combats were normally terminated at the end of 5 minutes, since it was found that the decision reached at the end of 5 minutes usually was that which obtained also at the end of a 20-minute period.

The result of an encounter is classified as "active" when both mice fight and one mouse finally submits. A "draw" is an active fight in which neither mouse submits before the time allotted for the encounter has elapsed. A "passive" victory is one in which one member of the pair attacks and the other mouse submits or runs away repeatedly. Encounters classified as "double passive" are those in which one mouse submits in spite of the fact that the other mouse has not attacked. The possible significance of such action will be discussed later. "No battle" is the term which has been used for an encounter in which neither mouse is aggressive, in which neither gives the submission reaction, or in which they remain inactive, may sniff and groom each other, or may wander about the cage.

Submissive behavior, as described by Ginsburg and Allee (1942), includes only the submissive stance in which a mouse rears rigidly on his hind legs, draws one foreleg close to the body, extends the other stiffly, and remains motionless; often this posture is accompanied by characteristic squeaking. It is quite different from the poised position assumed in preparation for an attack or an active defense. Mice also submit by repeatedly running from an opponent and squeak-

ing. This may occur when mice appear to have become so submissive that they are not able to remain near another mouse long enough to give the submissive stance. Uhrich (1938) also recognized such behavior as being submissive.

In tabulating the results of encounters each active or passive victory was given equal weight and recorded as one encounter won. This procedure gives only a rough approximation. Estimating the comparative value of different encounters presents difficulties. Contacts of the same kind may have different values at different times. The effect of winning one fight at a critical time may be more important psychologically than the winning of many similar fights later in a conditioning series. Double passive victories, however, were recorded as one-half unit, since the winning mouse did not attack. A "round" refers to a group of encounters in which each mouse in the experiment meets every other mouse in the group. The randomly selected lot of animals that were fought against each other were arbitrarily called a "set," or "group," of mice, despite the fact that they were not caged together.

The activities constituting a typical fight pattern, as described by Ginsburg and Allee (1942), were verified. In addition, an activity, termed "mincing," that is especially characteristic of the black mice, should be added. A mouse "minces" by taking many small steps in rapid succession near, or in circles around, his opponent. Such action may precede, accompany, or follow tail-lashing.

Three types of experiments were used to test the effect of the presence or absence of thiamin in the diet on the aggressive behavior of these mice. (1) Two experiments involved the addition of thiamin to a diet already adequate in this vitamin. (2) Two experiments were performed with one group of five mice and one group of four mice, each group of which did not receive any thiamin for 15 days previous to the initial encounters. (3) There were twelve experiments in which one or more mice were deprived of thiamin after the social order had been established; one was performed with a group of eight mice, one with a group of five, seven with groups of four, and three with groups of two mice. This last type of experiment will be referred to as "Type III." In addition, there were two control experiments in which a complete diet—either Fox Chow Checkers or our prepared diet plus all vitamins—was fed throughout.

In experiments of Type III, series of rounds were continued until the dominance hierarchy was established and was being repeated with a fair degree of regularity. The group was then shifted to the vitamin-controlled diet prepared by us, and the alpha mouse or, in some cases, more than one of those high in the social order (see Table 1) was deprived of vitamin B_1. The diet was continued until the mouse (or mice) not receiving B_1 had lost approximately 30 per cent of his weight (see Table 3), was down to about 19 or 18 gm. in weight, or until he was in such a weakened condition that he could barely move around. If our mice were permitted to drop a gram or two lower, they seldom could recover and soon died, even though replaced on an adequate diet. After stopping a thiamin-deficient diet, all the mice in the given experiment then received the same food, either the prepared diet plus vitamin B_1 or Fox Chow Checkers; and the encounters were normally continued until the social order present at the end of the test period had remained stable for several rounds or until another social order appeared and remained fairly constant.

In all but the first few experiments, when the importance of weights was not

fully realized, the mice were regularly weighed immediately after each group of staged encounters. During the period when they were being fed the experimental diet, they were weighed much more frequently.

The normal diet consisted of Purina Fox Chow Checkers, given daily in excess of the amount normally consumed. Unless otherwise stated, water was always available. The percentage composition of the experimental diet was as follows: sucrose, 55; vitamin-free casein,[2] 30; Osborne and Mendel (1919) salt mixture, 7; hydrogenated cottonseed oil,[3] 5; and cod-liver oil, 3 (Sandza and Cerecedo, 1941). The dry ingredients were thoroughly mixed in an electrically rotated ball mill; the melted cottonseed oil and cod-liver oil were added, and the whole diet was completely mixed again in the ball mill. Five grams of this diet were given daily to each experimental mouse, with certain exceptions, which will be noted later. All mice on the experimental diet had the following vitamins added daily to their food: 10 γ of riboflavin, 20 γ of pyridoxine, and 30 γ of dextrorotatory calcium pantothenate.[4] All mice except those in the alpha position and a few others listed in Table 1 received, in addition, 5 γ of thiamin (Merck's thiamin hydrochloride) daily. The amounts of thiamin, riboflavin, and pyridoxine necessary per mouse per day

TABLE 1

SUMMARY OF PROCEDURE FOR EXPERIMENTS IN WHICH MICE WERE DEPRIVED OF THIAMIN

Set	No. of Mice in the Set	Mice Deprived of Thiamin	Social Position	No. of Days on Diet	Frequency of Encounters
I	8	No. 6	alpha	24	One per mouse three times a week
		No. 1	beta		
		No. 8	gamma		
		No. 3	delta		
		No. 7	omega		
II	5	No. 15	alpha	25	Round-robin two times a week
		No. 13	beta		
III	4	No. 19	alpha	23	One per mouse three times a week
		No. 16	omega		
V	4	No. 31	alpha	24	Round-robin two times a week
		No. 29	beta		
VI	4	No. 33	alpha	25	Same
XIV	4	No. 78	alpha	18	Same
XIX	4	No. 95	alpha	26	Same
	4	No. 96	alpha	27	Same
	4	No. 96	alpha	26	Same
XXII	2	No. 126	alpha	21	One per day 6 days a a week
XXIII	2	No. 130	alpha	21	Same
XXIV	2	No. 132	alpha	21	Same

[2] Labco.

[3] Crisco.

[4] Riboflavin, pyridoxine, and d. calcium pantothenate were dissolved in 0.1 N HCl except during the period when vitamins were given as a liquid instead of being added to the food. During this period these three vitamins were dissolved in a solution of 25 per cent ethyl alcohol and 0.05 N HCl. At all times thiamin was dissolved in a solution of 25 per cent alcohol and 0.05 N HCl; the maximum amount of alcohol ingested per animal per day was 0.062 gm.

were based on the experience of Foy and Cerecedo (1941); the amount of d. calcium pantothenate is that recommended by Sandza and Cerecedo (1941).

Sometimes the mice did not eat all their food, especially those that were not receiving vitamin B_1; such mice gradually ate less and less, and on some days no additional food was given. However, under such conditions the above-mentioned vitamins were added daily to the food that remained in the dishes.

The prepared diet was placed in porcelain dishes, 2 inches high by $3\frac{1}{2}$ inches in diameter; these possessed a centrally directed $\frac{1}{2}$-inch overhang that prevented the mice from scattering the food. Five grams of food per individual was chosen as a more than adequate amount to be given daily, since no mouse ate more than one Purina pellet per day, the average weight of which was 4.7 gm. Four grams of Purina Fox Chow Checkers contain 22 micrograms of thiamin. Each dish and cage was plainly marked with the number of the mouse to which it belonged, so that mistakes in the distribution of the dishes of food and mice would not occur.

This study was in progress for over $2\frac{1}{2}$ years and includes twenty experiments involving 2,165 pair-contacts. The first author had complete care of the mice, including the daily feeding and the cleaning of cages except during annual vacation periods or for other brief absences from the laboratory. At such times they were cared for by someone skilled in studying animal behavior. She also conducted all fights, wrote the original records, and made the basic compilation of the crude data. The second author was in close touch with the work at all times and had much to do with both the general and the detailed planning of the work. We are jointly responsible for the final analysis and conclusions.

PRELIMINARY EXPERIMENTS

TEST OF FIGHTING TECHNIQUES

A group of eight mice was fought for 8 months in round-robin fashion. Every mouse necessarily met seven other mice in each round—a total of twenty-eight encounters per round. In this first set an entire round was not fought on a single day, since seven encounters per day were found to be too many to allow continued active fighting. The number of encounters per day and per week was varied many times during the course of this experiment in an attempt to discover an optimum. Fights were staged in which each mouse fought (1) once a day; (2) twice a day; (3) once or twice a day in many different sequences; and (4) one fight per mouse on three days of each week. This one experiment lasted for eighteen rounds, with a total of 461 pair-contacts.[5]

Eight mice made too large a group to handle conveniently and was not attempted again. With a group of four or five, or fewer, an entire round-robin could be run on a single day. In subsequent experiments, therefore, round-robins were usually staged twice each week at alternate 3- and 4-day intervals. Our mice would not continue fighting vigorously if more frequent encounters were arranged for them. The original group of eight was later used in the first experiment concerned with the effect of a deficiency of thiamin on their social order.

TEST OF ADEQUACY OF OUR FULL EXPERIMENTAL DIET

A preliminary test indicated that young C57 male mice grow slightly faster when fed Fox Chow Checkers than their littermates do when given our complete, vitamin-rich diet. On the other hand,

[5] Because of the deaths of two mice, there were only 461 encounters instead of the possible 504.

adult mice, such as were used in the dietary experiments, throve better on the latter diet. Some of the available data that support this statement are presented graphically in Figure 1 and 2.

Figure 1 shows that the average weights of nine adult C57 males that received the complete diet were greater than the average weights of twelve mice of comparable ages that received Fox Chow Checkers. The weights of two mice, Nos. 93 and 94, are given in Figure 2. During the periods inclosed in brackets these mice received the prepared diet plus vitamins A, D, thiamin,

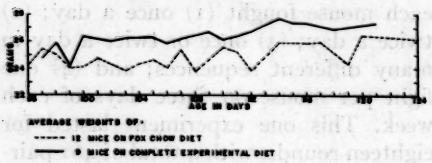

FIG. 1.—Average weight of twelve mice on a diet of Fox Chow Checkers and of nine mice of comparable ages on the complete experimental diet; all were black (C57) adult males.

riboflavin, d. calcium pantothenate, and pyridoxine—the complete diet. At all other times the mice were fed Fox Chow Checkers. It is evident that, while these mice were eating the prepared diet, their weight was maintained at, and in some cases exceeded, the weight which obtained at the beginning of each dietary period.

These experiences, together with many more not summarized here, indicate that the prepared diet was entirely adequate when vitamin B_1 was present. It follows that weight losses suffered by mice on our thiamin-free diet resulted directly or indirectly from the absence of thiamin, not from some other dietary deficiency.

ADDED THIAMIN

There is a widespread current belief among the lay public in the United States that, almost regardless of diet, added thiamin will increase the mental and neuromuscular proficiency of man. Some support for this viewpoint is offered by the work of Gounelle (1940); of Williams, Mason, Wilder, and Smith (1940); and of Simonson, Enzer, Baer, and Braun (1942). On first reading by a nonspecialist in human nutrition, the experiments of Harrell (1943) would appear to lend support to this belief. Her results will be discussed more fully later (p. 218). In order to provide a reasonably sound basis for interpretation of the results of experiments on thiamin deficiency, we made tests designed to answer the question: Will thiamin in excess amounts given to mice low in their social order that are on an adequate diet cause them to rise in social status?

After the original group of eight mice had been fought for eight complete rounds, mice Nos. 4 and 5 were at the very bottom of the social order. Mouse No. 4 had held this position since the fights were begun 73 days previously; No. 5 had won only one passive victory, and that was with No. 4 during the first round. Additional vitamin B_1 dissolved in 0.05 cc. of acidulated ethyl alcohol (p. 200) was given to Nos. 4 and 5; and an equal amount of acidulated alcohol was given to Nos. 1, 2, 3, 6, 7, and 8 for the same period, which lasted for 84 days. Mice can safely take more than 2 cc. of 35 per cent ethyl alcohol daily with no deleterious effects on health or growth (Nice, 1912). The maximum amount of alcohol received by mice in any of these experiments was less than one-tenth the amount of alcohol contained in 2 cc. of 35 per cent ethyl alcohol.

All eight mice were fed Fox Chow Checkers during this experimental period. Two methods of giving the extra thiamin were employed: (I) each day for 34 days vitamin B_1 was allowed to soak

into a depression scraped in the food; and (II) for 50 days it was given in solution in a glass tube that was suspended from the side of the cage. During this second period all water was removed from the cages until the solutions containing alcohol and water with or without vitamin B_1 had been consumed.

During 17 days (one full round) 3 γ of thiamin were given daily (Method I) to mice Nos. 4 and 5; they remained at the bottom of the order throughout this period. They received twice as much additional thiamin (Methods I or II) per day, or 6 γ, during the next 50 days and every Monday, Wednesday, and Friday for a period of 183 days.

After seven complete rounds, mice Nos. 16 and 18 had been at the bottom of their social gradient for five rounds and were selected to receive excess thiamin. The alpha mouse at this time was No. 17; he had won all his encounters for the previous five rounds. Mouse No. 19 held the beta position by virtue of winning all encounters for five rounds except those with No. 17. The other two mice, Nos. 16 and 18, received 3 γ of extra thiamin by Method I for one full round that lasted for 7 days and remained at the bottom

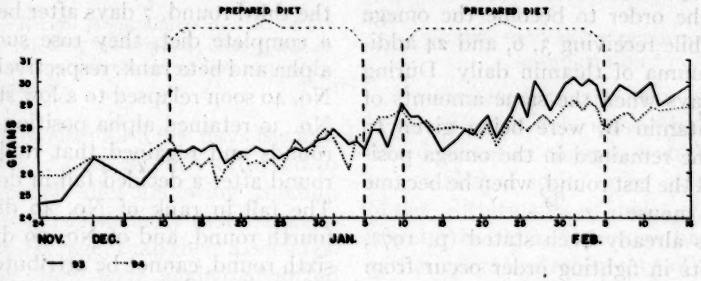

FIG. 2.—Maintenance of weight of the same C57 adult male mice on the complete prepared diet (between brackets) and on Fox Chow Checkers. See also Figs. 5 and 6.

remained in the omega position during three complete rounds of staged encounters.

These two mice continued to occupy the lowest position in the social scale for a final full round that lasted for 17 days. During this time they received 12 γ of thiamin per day (Method II) in addition to that present in the Purina Checkers. Thus the addition of excess thiamin to the diet of these two mice at the bottom of their social order did not cause them to rise in social position.

A repetition of this experiment was performed with four littermate males that had been isolated for 30 days before encounters were begun. Each mouse met only one other mouse in one encounter of their social order. Then during seven full rounds, which took place during the next 50 days, 6 γ of vitamin B_1 were given daily to the low-ranking mice—by Method I for 9 days, by Method II for 41 days. Of these, No. 18 remained in the omega position for the entire time. Mouse No. 16, on the other hand, after sharing the omega position for one round, then shared the alpha position with No. 19 for two rounds, in which they both won two encounters. He became, and remained, the beta mouse for three rounds and held the alpha position in the seventh round by winning one active and two passive encounters.

Immediately following this experience the added daily thiamin dosage was in-

creased to 24 γ for Nos. 16 and 18 for 22 days, in addition to Fox Chow Checkers; as usual, the others received the same basic diet plus an equivalent amount of alcohol and water. Three rounds of encounters took place in this period; No. 16 remained in the alpha position for two more rounds but fell to omega in the last round. Mouse No. 18 remained at the very bottom of the social order until the last round, when he won one passive victory and fought a draw to win the rank of beta mouse.

Thus No. 16 began in the omega position, shared alpha rank, dropped to beta, became alpha again, and fell to the bottom of the order to become the omega mouse while receiving 3, 6, and 24 additional gamma of thiamin daily. During the 79 days when the same amounts of added vitamin B_1 were being given to No. 18, he remained in the omega position until the last round, when he became the beta mouse.

As has already been stated (p. 197), such shifts in fighting order occur from time to time for reasons that have not been analyzed. We see nothing in the evidence at hand to indicate that the addition of thiamin to an already adequate diet increases either the aggressiveness or the ability of these C57 males to win in pair-contacts.

THIAMIN DEFICIENCY PREVIOUS TO ADULT SOCIAL CONTACTS

In order to separate the results of thiamin deficiency from concomitant social conditioning, two arbitrarily selected members of each of two experimental sets of mice were deprived of vitamin B_1 for the 15 days, preceding their first staged encounter. For the sake of brevity these will be spoken of as the "minus B_1" mice. Their associates—three in one set and two in the other—received the full, prepared diet, including thiamin, for the given 15-day period. These will be referred to as the "plus B_1" mice. Each entire set was returned to Fox Chow Checkers on the day that encounters were begun, and all received that diet thereafter. Each of the "minus B_1" animals lost weight during the time they were deprived of thiamin. The loss ranged from 2.8 to 12 per cent; in the first set No. 39 lost 6.6 per cent, and No. 40 lost 12 per cent, of his original weight.

As may be seen in Figure 3*a*, Nos. 39 and 40, the "minus B_1" mice of the first of these experimental sets, were at the bottom of the rank-order during the first two rounds (4 days) of fighting. During the third round, 7 days after being given a complete diet, they rose suddenly to alpha and beta rank, respectively. Mouse No. 40 soon relapsed to a low status, but No. 39 retained alpha position for three rounds and regained that rank for one round after a decided fall in dominance. The fall in rank of No. 40 during the fourth round, and of No. 39 during the sixth round, cannot be attributed to any change in weight. After twelve rounds (38 days) the most dominant of the "plus B_1" mice was clearly in the alpha position; little resistance was shown to him, and there was only slight tension apparent between the subordinate mice.

The record made by a second set of four mice given similar treatment supports these results even though neither of the "minus B_1" individuals rose to top rank in the upsurge that followed soon after their return to a diet containing thiamin. This test lasted for eight rounds, covering a period of 25 days. In place of giving the details of this repetition of the experiment, the general pattern of the results can best be seen by the lines summarizing the results of these two experiments which are shown in Figure 3*b*. The solid line gives the mean numbers of encounters won, round by round, for the

five "plus B_1" mice; and the broken line similarly records the mean status of the four "minus B_1" animals.

The "minus B_1" mice ranked far below their associates in the first two rounds of encounters—the first social contacts these mice had experienced since weaning. Then a marked upswing took place that is shown in the record of each

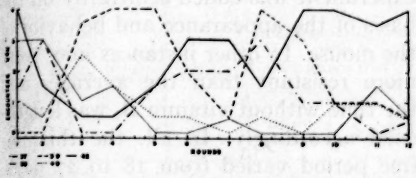

FIG. 3*a*.—Record of encounters in a set of five mice now on full diet, two of which, Nos. 39 and 40, did not receive any thiamin for 15 days previous to the first encounter.

individual mouse; for a few days the animals that had been recently returned to a thiamin-rich diet following thiamin starvation were decidedly more successful, on the average, than their associates. Thereafter, they were again outranked by the "plus B_1" mice.

It is difficult to interpret this whole experience with certainty. From the nature of the experiments we do not know the relative initial aggressiveness and fighting ability of any of these mice. From the results as they stand, it would seem that, regardless of initial qualities, the thiamin starvation probably had a depressing influence and that the return to a thiamin-rich diet was definitely stimulating. The results shown in the last eight rounds may be explained variously. There is a possibility that (*a*) the "minus B_1" mice tended to be innately less aggressive, perhaps even from the start; (*b*) these mice may have been innately more aggressive but experienced a hangover of ill-effects from the period of thiamin starvation; or (*c*) they may have been innately more aggressive, as shown by the taking of higher rank after return to an adequate diet, but later lost status because of unfavorable conditioning as a result of initial defeats when not yet in good physiological condition. Since we do not have the needed data on which to base a judgment as to which of these (or other) possibilities may have been effective, we are left with the upswing in social status following return to a good B_1 diet as the only positive result of the experience. The extent and the uniformity of the observed upswing seems significant despite the small sample and the known tendency for changes in fighting rank to occur for unanalyzed reasons.

THIAMIN DEFICIENCY AFTER SOCIAL STABILIZATION

Twelve experiments were performed in an effort to determine what effect a diet deficient in thiamin would have on an established social order among mice. Males—littermates or males of approximately the same age—were brought together in round-robin sets of pair-contacts until the social hierarchy appeared

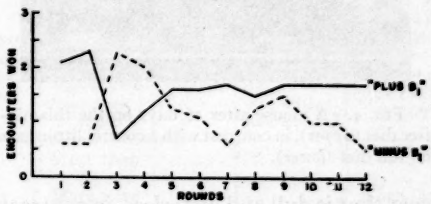

FIG. 3*b*.—Mean record of social status of nine mice following thiamin deficiency (*broken line*) for four of them.

to be fairly stable. Then mice were deprived of thiamin in nineteen instances. They ranked as follows when transferred to a thiamin-free diet: 12 alpha, 3 beta, 1 gamma, 1 delta, and 2 omega. Thiamin deficiency was continued until each animal had lost about 30 per cent of his weight or until he presented typically the appearance of mouse No. 169 (Fig. 4,

upper), in contrast with No. 171, a normal littermate (Fig. 4, *lower*).

At the time these pictures were taken, No. 169 had been deprived of thiamin for 26 days. He weighed 19.1 gm.—a loss of 35 per cent of his original weight. Meantime No. 171 had maintained his starting weight of 29 gm. during an equal length of time on the complete experimental diet. The thiamin-deficient mouse can be seen to have a rough unkempt

FIG. 4.—A mouse after 26 days on the thiamin-free diet (*upper*), in contrast with a control littermate on full diet (*lower*).

coat that is dull and lusterless, in contrast with the sleek, shining appearance of the normal animal. The deficient mice assume a humpbacked posture, often more marked than is shown in Figure 4. They also are more nervous than are normal animals and may become so weak that they move about with difficulty. If the weight of mice such as we were using fell below 18 gm. or if they showed avitaminosis to a marked degree, it was difficult, and sometimes impossible, to get them to recover when offered a thiamin-rich diet. We did not try injections of thiamin.

Some mice are affected more rapidly than others; they may show extreme signs of avitaminosis before losing even approximately 30 per cent of their weight. Mouse No. 78 (Table 3) was the only one of our experimental mice that behaved plainly in this fashion. In this case the experiment was ended arbitrarily on the basis of the appearance and behavior of the mouse. In other instances mice were more resistant than the average, and the time without vitamin B_1 was lengthened accordingly. In all, the thiamin-free period varied from 18 to 27 days.

A few selected experiments will be reviewed briefly before attempting a more general analysis.

The first experiment in this series was with the original group of eight mice in Set I (p. 291). Six days after Nos. 4 and 5 were changed from excess amounts of thiamin to the standard diet, five of the other mice of the set were deprived of thiamin for 24 days. Some of the details of the experimental results are given in Table 2. Of those deprived of thiamin, No. 6 was the alpha mouse—a position recently held by No. 8, now in third rank. Mouse No. 1, now beta had steadily ranked beta or gamma for 3 months. In addition to these three high-ranking mice the following individuals also were put on the thiamin-free diet: No. 3, tied for delta position although formerly he was the alpha mouse of the lot; and No. 7, one of the omega animals, usually intermediate in rank.

Mouse No. 6 continued to win in all his pair-contacts while without vitamin B_1 and for the 42 days that followed. As may be seen in Table 3, No. 6 is one of three alpha individuals that maintained top rank; No. 96 did so in two successive experimental periods. It is possible that No. 6 was not deposed as soon as some

other alpha mice under similar dietary deficiency in other experiments because he was given fewer pair-contacts per week. Mouse No. 6 met other mice only once on any given day, and then only on three days per week. He did not have the opportunity to become tired by fighting, and neither did he have as many chances to submit as did the alpha mice in other experiments. Normally these others met all mice in their set, round-robin fashion, twice each week.

TABLE 2

EFFECT OF THIAMIN-FREE DIET ON WINNING FIGHTS

Mouse No.	Thiamin	Fights Won							
		Just before Diet		Mid-diet		Just after Diet		Later	
		Percentage	Rank	Percentage	Rank	Percentage	Rank	Percentage	Rank
1	−	86	2	43	4	0	.....	0*	.....
2	+	25	4	0	.....	0	.....	0	.....
3	−	25	4	50	3	16	3	0	.....
4	+	0	.....	0	.....	8	5	0	.....
5	+	0	.....	14	6	33	2	80	2
6	−	100	1	100	1	100	1	100	1
7	−	0	.....	86	2	16	3	0†	.....
8	−	71	3	43	4	0	.....	0	.....

* Died 1 day after end of diet.

† Died 6 days after end of diet.

TABLE 3

EFFECT OF THIAMIN DEFICIENCY ON MICE IN ESTABLISHED ALPHA RANK

Alpha Animals Deprived of Thiamin (Mouse Nos.)	No. of Mice in Set	Days in Alpha Position before Diet	Days on Diet	Percentage of Period in Alpha Position	Percentage of Weight Lost at Fall in Rank or at Close of Thiamin-Free Diet	Weight at Fall (Grams)	Percentage of Difference between Weight of Alpha Mouse at Fall and Rest of Group	Percentage of Weight Lost by Alpha Mouse at End of Diet
6*	8	5	24	100	More than 16.2	.....	.....	.....
15	5	74+30†	25	92	More than 25.8	.....	.....	More than 35.3
19	4	15+31+1†	23	65‡	.....	.....	.....	.....
31	4	25	24	92	25.6	19.4	−22.5	31.8
33	4	28	25	68	20.2	21.0	−23.4	35.4
78	4	8+28†	18	66‡	6.9	21.8	−16.7	14.5
95	4	4+31†	26	31	2.8	29.5	+14.2	37.0
96	4	23	27	100§	39.1	17.7	−39.6	39.1
96*	4	8+13†	26	100	30.1	19.8	−38.0	30.1
126*	2	21	21	95‡	17.1	19.1	−33.3	17.1
130	2	20	21	67	12.4	22.0	−23.9	29.5
132	2	23	21	86	21.7	20.6	−28.4	25.4

* Continued in alpha position after end of diet.

† These mice had held alpha rank for more than one period. The last figure gives the days in alpha position immediately before being deprived of vitamin B_1.

‡ Dropped from and returned to alpha position while on diet.

§ None of these mice showed any aggressive action on the last day of the diet; No. 96 was clearly in alpha rank 3 days before and 4 days after that time.

Since the importance of frequent weighings was not then realized, the weight of No. 6 was recorded 3 days before the diet ended, not at the end. At this time he had lost more than 16 per cent of his original weight. Later experience showed that between 2 and 3 gm. usually are lost during the last 3 days on a thiamin-free diet; thus, No. 6 maintained alpha status after having presumably lost approximately 24 per cent of his weight.

Of the other mice on the thiamin-free diet, as shown in Table 2, two (Nos. 1 and 8) showed a steady decrease in percentage of contacts won; of the two others, No. 3 increased its percentage won and then declined to a multiple tie for lowest rank; No. 7 followed the same course but more spectacularly. Of those on a complete diet throughout, only No. 5 showed a steady advance in rank, and in the postdietary period he defeated all his associates except the dominant mouse, which had been on a thiamin-free diet.

In summary, three trends are revealed: (1) the alpha individual retained his rank, with or without thiamin. (2) His four associates without thiamin lost rank either steadily or after an initial gain. (3) One of three mice receiving thiamin throughout rose to beta rank; the other two remained low in social rank.

The extent of physical weakening induced by lack of thiamin is shown by the death of No. 1 on the day after being returned to a full diet and by the death of No. 7 on the fifth thereafter. These and other deaths following lack of thiamin are recorded in Table 4. Since No. 6 remained in alpha position with treatment identical to that given Nos. 1 and 7, and in consideration of the other tendencies just summarized, one is tempted to conclude immediately that, until athiaminosis is well advanced, presence or absence of vitamin B_1 in the diet of a previously well-fed mouse is of subordinate, rather than prime, importance in winning and holding social status.

TABLE 4

DEATHS RELATED TO LACK OF THIAMIN

Mouse No.	Weight at Death (Grams)	Weight Lost while Thiamin-starved		Time of Death
		Grams	Percentage	
1	18.2	10.8	37.2	1 day after return to thiamin
7	22.0	11.7	34.8	6 days after return to thiamin
13	22.5	11.3	33.5	6 days after return to thiamin
16	19.4	.....	.....	5 days after return to thiamin
19	17.2	.....	.....	9 days after return to thiamin
29	15.7	10.1	39.2	1 day before return to thiamin
33	17.2	9.1	34.6	1 day before return to thiamin

Another group of four mice—Nos. 29, 31, 32, and 36*a*—met in a complete round-robin of staged encounters each Tuesday and Friday, for a total of thirty rounds. The alpha rank was held for the first three rounds by No. 29, and by No. 31 for the next seven. At the beginning of the eleventh round the group was placed on the experimental diet and No. 31 was deprived of thiamin, as was No. 29, which had occupied beta rank for six of the seven rounds since he had dropped from the alpha position.

While on the thiamin-free diet, No. 31 remained in top rank for 22 days before dropping to beta position, or for 92 per cent of the experimental period. At this time he weighed 22.5 per cent less than the average weight of the other mice in the group and had lost 25.6 per cent of his weight.

Mouse No. 31 was replaced in the alpha position by No. 32, which just previously had been at the very bottom of his order and which weighed 11.1 gm. more than did No. 31 at this time. In twelve of the thirteen rounds that were staged after the diet was ended, No. 32 retained his high rank except for a brief period, when No. 31 again momentarily held this position.

Mouse No. 29, also deprived of thiamin, dropped from beta to gamma position during the first round on the experimental diet but regained his former status during the second and third rounds. Although he exhibited no apparent aggressive action during the next three rounds, he was submitted to in the fourth round by No. 36*a*, consistently an omega mouse, and in the sixth by No. 32. Mouse No. 32 weighed 10.52 gm. more than did No. 29 at this time; the latter had lost 24.4 per cent of his weight since the experimental period began. Two days before the mice were to have been returned to a complete diet, No. 29 shared the omega position with No. 36*a*. On the next day he died. He had lost 39.2 per cent of his weight in the 23 days during which he had been deprived of thiamin.

Three experiments were performed in succession on one group of four littermate males—Nos. 93, 94, 95, and 96. After 94 days of isolation these were brought together in complete round-robins twice each week. Although no aggressive action was evident during the first or third rounds of encounters, No. 95 fought one draw with No. 96 during the second round, received one passive submission by No. 94, and one double passive (see p. 198) submission by No. 93.

At the beginning of the twelfth round, the alpha rank had been continuously held for eight rounds (28 days) by No. 95; he had won all his encounters in each round. The other three mice had jointly occupied the omega position for the last seven rounds. At this time the group was placed on the experimental diet, and No. 95 was deprived of thiamin for 26 days—a period that included seven rounds.

Mouse No. 95 remained in the alpha position for only 8 days (two rounds). During the third round, in which both Nos. 95 and 96 won two encounters, No. 96 beat the former in an active battle, in addition to winning an encounter with No. 93. Mouse No. 96 thereafter took highest rank among these four mice and won all his encounters in the remaining five rounds that were included in the experimental period. Except for one passive victory in the fourth and in the sixth round, No. 95 did not win any additional encounters. Mice Nos. 93 and 94 did not exhibit any aggressive behavior during the entire experimental period.

When No. 95 was deprived of thiamin, he weighed 4.5 gm. more than the average weight of the other mice in the group ($\frac{1}{2}$ gm. within the weight difference listed by Ginsburg and Allee [1942] as giving significant advantage to a mouse). He weighed 14.2 per cent more than the average weight of the other members of the group when his drop in rank took place, and he had lost only 0.86 gm. while not receiving thiamin. Mouse No. 95 is the only alpha mouse in this series of experiments that fell from the top rank while weighing more than the average of the other members of the set. It is highly probable that this shift in dominance

was only slightly, if at all, affected by the fact that No. 95 was receiving no thiamin; it appears to be an instance of one of the changes that occurs from time to time for reasons that we do not understand. By the end of the 26-day period, No. 95 had lost 37 per cent of his original weight. He regained this weight during the 7 days following this experiment, when the mice were being fed Fox Chow Checkers. In the two rounds that took

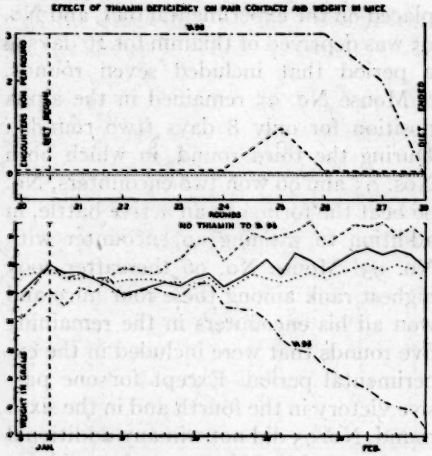

FIG. 5.—Effect of thiamin deficiency on pair-contacts and weight of mice.

place in this interval of time, No. 96 remained the dominant mouse and the others jointly held omega rank.

A second thiamin-free period began at this time, when the body weights of all four mice were between 27.3 and 29.0 gm. Since No. 96 was the dominant mouse, he was placed on the thiamin-free diet. As Figure 5 shows, he retained alpha rank and received submissions from his associates until the last day of the experimental diet, when he had been without thiamin for 27 days; on that day he attempted to attack No. 93 but was too weak; the others in the group did not show any aggressive behavior. In the meantime, No. 96 had lost 39.1 per cent of his weight and weighed 39.6 per cent less than the average weight of those mice that were receiving thiamin. Mice Nos. 93 and 94 exhibited no aggressive behavior during this 27-day period; No. 95 fought two active battles that were won by No. 96 and won one passive victory from No. 94.

The mice were given Fox Chow Checkers during the next 25 days—a period that included seven rounds. During the first of these rounds No. 96 won all three encounters, to become the alpha mouse again. This occurred only 4 days after the end of the previous experiment when he had attempted, but had been physically unable, to attack. Mouse No. 96 was less aggressive but still definitely held alpha rank in the following round; then, after winning none of his encounters in the third round, he won all in the fourth, fifth, sixth, and seventh rounds. There were minor changes in the social rank of Nos. 93, 94, and 95 in the first five rounds following the end of the previous dietary period. However, by the sixth round none of them were exhibiting aggressive behavior and No. 96 was readily obtaining submissions from all three. This again gave the usual two-rank mouse hierarchy (Uhrich, 1938) of one dominant and all the rest equally subordinate to him.

At this time No. 96 was deprived of vitamin B_1 for a second time. As shown in Figure 6, he maintained his alpha rank throughout the entire thiamin-free period of 26 days, winning all encounters (three in each of eight rounds). At the end of the experimental period he had lost 30.1 per cent of his weight and was 38 per cent under the average weight of the three mice that were on a complete diet. He retained dominance not only for the 26 days during the experiment but also for four additional rounds that

occurred in the 14 days after the termination of the diet and before this set of staged encounters was discontinued.

In the round after the group was returned to Fox Chow Checkers, No. 96 won all encounters except one listed as "no battle." Throughout this last phase of the experiment Nos. 93, 94, and 95 did not attack any other mice and continued jointly to occupy the omega position.

Perhaps these accounts of experiments on three different sets of mice give a sufficient insight into the methods used and some of the results obtained. Mice Nos. 6, 96, and 126 were unusual in that they remained at the top of their respective orders after being returned to an adequate diet. Mouse No. 96 might not have done so in his first deprivation of thiamin if he had been associated with a mouse that was then as aggressive as No. 95 had been a few weeks before. On the other hand, No. 95 illustrates the earliest fall from dominance while on diet. Only 31 per cent of the period had passed when No. 96 replaced him; the latter was then on the full vitamin diet.

Inspection of Table 3 shows that half of these alpha mice maintained that rank 90 per cent or more of the thiamin-free period and that only one was not at the top of its fighting order for at least 65 per cent of the period. Two mice, Nos. 19 and 78, returned briefly to alpha status while still without thiamin before falling to beta and gamma rank, respectively, at the end of the dietary period. Mouse No. 126, although retaining alpha rank, became passive near the end of his period of deprivation of thiamin but regained aggressiveness on the first day he received a full diet.

It will be recalled that, when mice were deprived of thiamin for 15 days before their first adult social contact (p. 204), they showed a strong upswing in percentages of contacts won after a few days on a full diet. Examination of the records in the present type of experiments reveals that, in all, nineteen mice were deprived of thiamin after their social rank was well established. Seven of these died, apparently as a direct result of the experience. Of the remainder, seven became more successful in combats shortly after being returned to a thiamin-rich diet; four—two of alpha rank and two of omega rank—continued in their previous

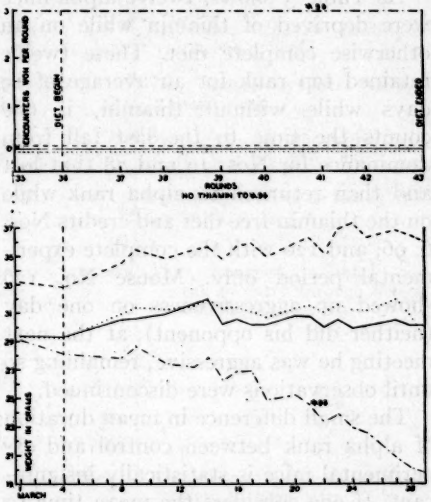

FIG. 6.—Effect of thiamin deficiency on pair-contacts and weight of mice.

rank, and one declined still further. In all, omitting those that died and those already at the top of their respective groups, seven of ten of these mice showed recognizable improvement in ability to win shortly after being returned to a complete diet after from 18 to 27 days of thiamin starvation. This supports the results reported on an earlier page (p. 205) without giving an explanation for them.

In all, we have had experience in this series of experiments with a total of twenty-three different mice of alpha rank among control groups of four or

more mice. The same mouse attained this status in some groups more than once; hence we have records for thirty-eight control periods. These mice remained at the top of their respective sets of associated individuals for an average of 19.2 days before losing alpha status to some other mouse for reasons unknown to us. We can conceal our ignorance of the factors underlying such changes by calling them "spontaneous."

As Table 3 shows, twelve alpha mice were deprived of thiamin while on an otherwise complete diet. These twelve retained top rank for an average of 19 days while without thiamin, if one counts the time to the last fall from dominance for Nos. 19 and 78 that lost and then returned to alpha rank while on the thiamin-free diet and credits Nos. 6, 96, and 126 with the complete experimental period only. Mouse No. 126 showed no aggressiveness on one day (neither did his opponent); at the next meeting he was aggressive, remaining so until observations were discontinued.

The small difference in mean duration of alpha rank between control and experimental mice is statistically insignificant. If one considers the mean time to first fall from complete dominance to be the better end-point, then the mean duration of alpha status while on a thiamin-free diet becomes 18 days and the difference between experimentals and controls is still statistically insignificant.

These time spans do not measure the full length of the maintenance of alpha rank by these particular mice, since each had been dominant in its group before being deprived of thiamin; and since three mice remained in alpha status until observations were stopped—42, 14, and 8 days, respectively, after the deficient diet ended. The measured alpha period for mice in groups of four or more —before, during, and after the thiamin-deficient diet—averaged 46.2 days, somewhat more than double the mean control period—a highly significant difference.

Some aspects of aggressive behavior persist after mice are no longer physically able to attack or fight. Table 5 shows that the alpha mice being considered continued to exhibit aggressive behavior for 14–27 days after having been placed on a diet deficient in thiamin. Three mice (Nos. 6, 96, and 126) even continued to be aggressive after being replaced on a complete diet. Mouse No. 96, although very thin and hunched—a condition typical of mice in advanced athiaminosis—made nine hard attacks and one chase against his opponents after having been deprived of thiamin for 26 days. In a previous experiment, No. 96 had attempted to attack when he was so weak that he tottered and shook when he tried to move. This occurred after he had been without thiamin for 27 days.

In addition to attacking and chasing, aggressive behavior may include sniffing, mincing, and tail-rattling. If the mouse being attacked is very submissive, he frequently will continuously run away from the dominant mouse, which will then chase him. Thus, chasing usually indicates that an extremely submissive mouse is one member of the pair. When mice are very aggressive, they usually attack without any preliminary sniffing. However, as they become physically weakened on a thiamin-deficient diet and unable to fight, attack, or run, the last evidence of aggressive behavior is usually shown in relatively innocuous behavior patterns directed toward another animal. They may merely sniff at, or attempt to groom, the other mouse; these activities are not always associated with aggressive behavior.

Mice sometimes may hold the dominant position after they have ceased to

exhibit observable aggressive behavior. Mice Nos. 15 and 132 were both dominant 3 days after they had last exhibited aggression, and No. 126 was the alpha mouse 2 days after the last aggressive behavior was seen. In such instances a mouse retained dominant rank without any observed aggressive action on his part; he received passive submissions from severely conditioned mice.

On the other hand, mice may exhibit some aggressive behavior when they no longer occupy the dominant rank. Mouse No. 33 dropped from the dominant position after having been without thiamin for 19 days. He was aggressive 3 days later, when he made two attempts to attack, and chased one mouse, although he was thin and hunched and wobbled as he walked. He was so weak that he could not carry through the attacks he attempted to make.

Mouse No. 95, the only one that dropped from alpha rank very soon after

TABLE 5

RELATION BETWEEN AGGRESSIVE BEHAVIOR AND THIAMIN DEFICIENCY

Mouse No.	No. of Days without Thiamin	Showed Aggression through Diet Day	No. of Days Dominant without Thiamin	Behavior on Last Aggressive Day while without Thiamin	Comments
6.....	24	24	24*	2 attacks	Aggressive behavior continued after end of diet
15.....	25	20	23	11 attacks 1 chase	Very weak 3 days after last aggressive day
19.....	23	19	15†	1 attack 1 tail rattle	Very sick, weak, hunched, 2 days after last aggressive day
31.....	24	22	22	3 attacks 1 fight 6 sniffs	Hunched, tottered on last aggressive day
33.....	25	22	19	2 attempts to attack 1 chase	Thin, hunched, wobbled; too weak to carry through attack on last aggressive day
78.....	18	15	12‡	2 attacks 1 fight 3 sniffs	
95.....	26	19	8	1 attack 1 attempt to attack	
96.....	27	27	27	Attempts to attack	Tottered and shook; too weak to carry through attacks; very hunched on last aggressive day
96.....	26	26	26*	9 hard attacks 1 chase	Very thin and hunched; aggressive behavior continued after end of diet
126.....	21	18	20*‡	3 attacks (1 hard)	Aggressive behavior continued after end of diet
130.....	21	14	14	5 attacks (1 hard)	
132.....	21	15	18	4 attacks	

* Continued to be aggressive after diet ended. † Lost, regained, and lost alpha rank. ‡ Lost and regained alpha rank.

being deprived of thiamin, showed some aggressive behavior 11 days later. He is probably the only alpha mouse in this series that lost dominant rank permanently for reasons other than those connected with physical weakening due to thiamin deficiency.

Comparison of the last two columns in Table 5 indicates that mice may behave aggressively when thiamin depletion has made them physically unable to attack effectively. Thus the thiamin deficiency we have been studying affects, in most instances, fighting ability, not aggressive behavior.

This is the ascertained evidence. Analysis and consideration of possible interpretations is reserved for the following section.

ANALYSIS AND DISCUSSION

Ideal experimentation would have required having two similar groups of mice, alike in every respect, ready for each test. The dominant mouse in one of these would then have been deprived of thiamin; and at the same time the other group, with a similarly dominant mouse, would have been held as a control. Such procedure was not practicable. Among the several possible alternatives, we chose to calculate the expected duration of continued dominance on the basis of the length of observed periods of alpha status for all comparable controls. The basic data are given in the first three columns of Table 6. Expected additional alpha days at any given time were then calculated by first subtracting the total number of days a given class of mice had remained in alpha position (col. III) from the total remaining number of alpha days for all the mice (col. IV) and then subtracting the number of elapsed alpha days for the mice that were still dominant (col. VI). This second remainder was then divided among the mice remaining dominant, to find the mean expectancy of continued dominance.[6] When smoothed by the usual five-point weighted formula, this calculated expectancy gives a base line against which to compare the duration of continued alpha rank of mice when deprived of vitamin B_1. The available data are so compared in Figure 7. It is difficult to draw exact conclusions from these twelve cases. The mean duration of complete dominance on a thiamin-free diet practically coincides with mean expectancy; the *P*-value of the difference is 0.8.

This means that, although nine of the top-ranking mice fell from alpha status when they were drastically affected by athiaminosis after having been on a thiamin-free diet for about 3 weeks, the normal control alpha mice would have had about the same expectancy of dominance.

Despite the similarity, amounting almost to statistical identity between maintenance of top rank without thiamin and expectancy based on experience with controls, we cannot escape the conclusion that there was a decrease in the fighting ability of the thiamin-deficient mice. The alpha mice which were deficient in thiamin continued to exhibit aggressive behavior, such as chasing, tail-rattling, and attacking, after they were physically very weak and actually unable to fight. Attempts to attack were made by some mice when they were so feeble that they could scarcely move. Thus it seems evident that in most cases physical ability to fight, and not aggressive behavior as such, is affected by lack of thiamin. The learned reactions of the associated mice were not affected, and submissions continued to be given by subordinates to alpha mice that could

[6] We are indebted to Mr. Asher J. Finkel for proposing this method of treatment and to Professor Sewall Wright for checking the method critically.

not have forced submission by active combat.

It is possible that there are aspects of aggressive behavior to which the mice react by submitting but which either are not apparent to us or are of such a nature that they cannot be detected by man. Mice have been seen to submit repeatedly to other mice which have not ruffled their hair, rattled their tails, minced, or made any observable movement, threatening or otherwise, toward the submitting animal. Since such submissions have occurred frequently in our experience, they have been considered as a response to some unobserved manifestation of aggressiveness or to memory of previous experiences, and the mouse to which the submission was made has been credited with a double passive victory.

In some instances mice have been conditioned downward in the social scale so forcibly and for such a long time that they react by submitting to noises, movements of the partition, etc., in addi-

TABLE 6

OBSERVED DURATION OF PERIODS OF DOMINANCE IN DAYS FOR CONTROL MICE TOGETHER WITH CALCULATED EXPECTATION OF CONTINUED DOMINANCE

Observed			Total Days of Alpha Rank *minus* Accumulated Values in Column III	Calculated		Expected Additional Alpha Days*	Smoothed Values†
No. of Individuals (I)	Days in Alpha Position (II)	I *times* II (III)	(IV)	No. of Mice Remaining in Alpha Position (V)	II *times* V (VI)	(VII)	(VIII)
Totals *and* mean			728	38	0	19.15	
7	4	28	700	31	124	18.58	18.6
2	5	10	690	29	145	18.79	18.6
1	6	6	684	28	168	18.42	18.8
1	7	7	677	27	189	18.07	19.5
6	8	48	629	21	168	21.95	20.5
3	11	33	596	18	198	22.11	21.0
1	14	14	582	17	238	20.23	20.8
1	15	15	567	16	240	20.43	20.6
2	18	36	531	14	252	19.92	20.8
2	19	38	493	12	228	22.08	21.2
1	21	21	472	11	231	21.90	20.7
1	24	24	448	10	240	20.80	19.7
1	31	31	417	9	279	15.33	18.1
2	32	64	353	7	224	18.42	17.7
1	36	36	317	6	216	16.83	17.7
1	37	37	280	5	185	19.00	18.5
1	41	41	239	4	164	18.75	18.4
1	43	43	196	3	129	22.33	17.7
1	56	56	140	2	112	14.00	13.5
1	66	66	74	1	66	8.00	8.3
1	74	74	0	0	0	0.00	0.0
Total *or* mean...38	19.5	728					

* $\frac{\text{IV } \textit{minus} \text{ VI}}{\text{V}}$.

† Smoothing formula $a + 2b + 3c + 2d + e$, with appropriate modifications at beginning and end.

tion to other mice that might possibly be showing "unobserved aggressive behavior." These may be unoriented fear reactions. Since, under our experimental conditions, it has been impossible to differentiate between submissions by mice that appear to submit to a variety of stimulations because of extreme conditioning and submissions to mice that may be exhibiting aggressive behavior not obvious to us, each such encounter has been recorded as a double passive victory in favor of the mouse to which the submissions were made.

The long duration of about the same period of expectancy of continued dominance among control mice is—at least in part—an expression of the effect of winning on continued winning; it is a partial measure of what may be called a "psychology of success" (Ginsburg and Allee, 1942; J. P. Scott, 1944). It is a matter of much interest that a mouse that has just beaten all its associates for the first time has a mean expectancy of dominance of slightly more than 19 days and that, after twice 19 days have passed, if still dominant, the mean expectancy of continued alpha rank remains approximately the same. This last expectancy must be taken with reservation, since it is based on only a few cases.

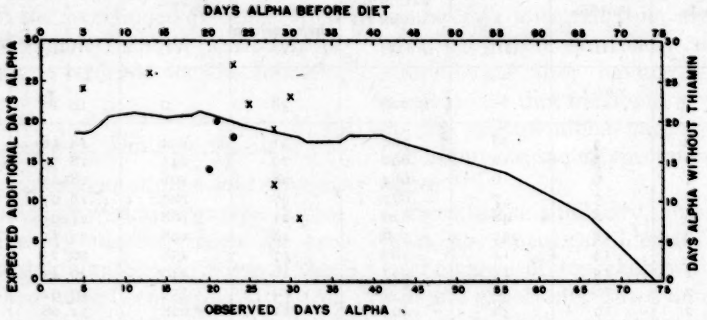

FIG. 7.—The solid line gives the calculated expectancy of continued dominance (ordinates to the left) for the different periods indicated on the lower abscissas. Observed days of continued dominance for mice without thiamin are spotted with abscissas at the top and ordinates at the right. The cross (×) indicates the observed dominance of the alpha mouse in groups of four or more; ⊙ gives similar values in groups of two mice.

The measure of the effectiveness of extended absence of vitamin B_1 from the diet may be estimated by a continued comparison with the expectancy curve shown in Figure 7. The alpha mice subjected to thiamin starvation had already been at the top of their respective groups for an average of 20.7 days when placed on the thiamin-free diet. After a vitamin B_1 starvation of 23 days or less, nine of the twelve had lost their alpha rank; and yet at this time (43 days) the controls still had a mean expectancy of continued alpha rank almost as great as the calculated expectancy at the beginning of this series of pair-contacts.

The continued dominance of our alpha mice before, during, and after being deprived of thiamin showed an average of 46.2 days for the mice fought in groups of four or more; this may be merely another expression of the same psychology of success. It is possible, too, that we selected unusually dominant mice to be placed on a thiamin-free diet. The evidence at this point is not conclusive. However, on the basis of the calculated points on our expectancy table, for the

time intervals used, it should not have made any difference how long an animal had been in alpha position when he was placed on the diet deficient in thiamin. Up to and including 43 days, a mouse has about the same chance of maintaining or dropping from alpha rank as he had after 4 days of alpha status. Thus it should make no difference that the twelve mice which we chose to put on the deficiency diet had occupied the alpha rank from 1 to 31 days. After the first experiment we consciously avoided withholding thiamin from mice that ranked generally low in winning ability, even though one such might at times be dominant. Otherwise, except with mouse No. 15 and perhaps No. 96, we are not aware of having selected for unusually strong dominance, although, as just stated, we did select away from weak dominance. There was no such selection among the controls; hence the calculated expectancy of continued dominance among the controls may be somewhat low.

After the hierarchy is established, the effect of conditioning on rank in these mice is probably closely similar, regardless of whether the alpha mouse continues to receive or is deprived of thiamin. In our usual type of experiment four more-or-less arbitrarily selected mice were caged separately and brought together in a long-continued series of pair-contacts. Although unexplained changes often occur, the winning mouse in such a group tends to continue to win, and the mice near or at the bottom of the given social order tend strongly to continue to lose. It is the whole group that is conditioned: one mouse to win over the others; one, perhaps, to win sometimes; and one or more, often three out of four, usually to lose. Depriving the dominant animal of thiamin does not alter the group behavior pattern as soon as athiaminosis becomes apparent, since the mice low in the social scale are not directly affected. Aggressive, strange mice, unaccustomed to being beaten, would probably defeat a thiamin-deficient mouse long before any member of his own group would break through his training not to attack an aggressor. Perhaps the underlings were trained not to attack a particular aggressor recognized as an individual. Our observations do not permit a decision on this point.

A general result of these experiments is the strong indication that there are two important factors affecting success in succeeding encounters among these mice. They are: (1) training in success of the accustomed victor and in lack of success of those that become accustomed to defeat (cf. Ginsburg and Allee, 1942) and (2) ability—in this case especially neuromuscular co-ordination and muscular strength. It appears that these two factors are at least partially independent and that sudden, complete withdrawal of vitamin B_1 from the diet affects the ability to fight without, so far as we have observed, altering either the statistical expectation of success by the dominant mouse or the conditioning not to attack on the part of subjugated animals.

Confusion exists in the literature on what constitutes an adequate daily amount of thiamin for different ages and conditions of man. According to National Research Council Standards (cf. Taylor, 1942, p. 54), children, 7–9 years old, are allowed 1 mg. of thiamin daily; young men, 16–20 years old, require double that amount. Keys and Henschel (1942) report that more than 1.7 mg. of thiamin chloride daily is not beneficial to healthy young men expending an average of 3,700–4,200 calories per day. Until such confusion is resolved, one cannot determine whether a given increase in thiamin constitutes "supercharging" or

is merely bringing a diet up to the optimum needed for best performance. For example, the interpretation of the careful experiment of Harrell (1943) on the effect of added thiamin on school and other performances of children and young people under well-controlled conditions depends on whether the amount of thiamin received daily in the normal diet (0.6–1.3 mean, 0.9 mg.) was adequate or inadequate. Harrell did not assay urinary thiamin output of her subjects; hence we have no indication of the adequacy of the basic diet except that furnished by the performance of the experimental subjects. Using National Research Council Standards, even when discounted 30 per cent for their factor of safety, the diet was low in thiamin; and the observed improvements, over and above those shown by the controls, would then be interpreted as resulting from having the thiamin level brought up into the range of normal adequacy. Harrell's human subjects, according to this assumption, were reacting as did our mice when returned to a normal diet after a period of thiamin deficiency.

Contrariwise, if Harrell's subjects were already receiving adequate thiamin for their ages and condition of life, as may be suggested by extrapolation from the work of Keys and associates (1942, 1943), then her experiments give evidence of beneficial effects from "supercharging." It should be remembered that extrapolation is usually hazardous, especially so in thiamin physiology when different age groups are being tested.

Gounelle (1940), who gave between 10 and 15 mg. of vitamin B_1 to eight racing cyclists, believes that the performance of the athletes was much better than might have been expected. Two football players, who had not played for several months, received the same large amounts of thiamin and were able to play without undue effort. Contrary to the experience of others, the exercise was not followed by days of fatigue or muscular lameness. Two women who had previously been on a diet which contained only 0.15 mg. of thiamin per day were, according to Williams, Mason, Wilder, and Smith (1940, p. 795), "strikingly more alert and attentive, and their performance with the chest weight exercise was much more satisfactory than it had been originally" when the daily intake reached 2 mg. a day; they found some evidence that 0.95 mg. per day relieved evidence of deficiency. Since these results are based on only two subjects and since influence of training and of the menstrual cycle were not considered, generalizing conclusions as to the effect of excess thiamin cannot safely be drawn.

Keys (1943), in reviewing a large part of the extensive literature concerning physical performance in relation to diet, pays due attention to the possibility of securing beneficial results from so-called "supercharging" with vitamin B_1 and other members of the B complex. He concludes (p. 170): "It would seem that there is at present no basis for high supplementation with vitamins of the B complex to promote better work performance." His own experiments support this conclusion. Our mice showed no improvement in ability to win encounters when given thiamin in addition to that present in a diet that was known to be adequate in respect to this vitamin. In this regard, results with these mice resemble those obtained by Keys, Henschel, Mickelsen, and Brozek (1943) with normal healthy young men. They concluded that such men, eating about 3,000 calories per day, are not benefited in various work tests, including simple strength, speed, coordination, and in glucose tolerance by a thiamin intake of more than 0.23 mg. per 1,000 calories per day over a period

of three months. This is less than Harrell's experimental subjects received per 1,000 calories per day in their basic diet. Simonson, Enzer, Baer, and Braun (1942) also found that a surplus of vitamin B complex had no influence on performance in five different types of muscular work. However, they decided that (p. 88) "vitamin B surplus improves the working capacity for occupations where fatigue is due to fatigue of the central nervous system. . . . ."

It will be understood that we have been studying the effect of thiamin upon aggressiveness and on fighting ability as revealed in mouse behavior. Our results indicate that thiamin is more important for maintaining physical vigor than for the maintenance of accustomed social status. We have not attempted to study the physiological mechanisms involved. The voluminous literature in the field reveals much evidence of various kinds of effects of thiamin deficiency on the peripheral and central nervous system of man and of other animals. Zimmerman (1943) cites and reviews much of this literature. According to Williams and Spies (1939), degeneration of the nervous system occurs in most, if not all, species suffering from prolonged inadequacy of vitamin B_1. Wintrobe *et al.* (1944) report opposed results for the pig; they found no nerve degeneration in chronic thiamin deficiency. They suggest that certain effects heretofore attributed to vitamin B_1 deficiency may, in fact, have resulted from lack of pantothenic acid.

Continued depletion results in death in various experimental animals. As Table 4 indicates, some of our mice died when deprived of thiamin for 23–25 days. Other mice under identical dietary conditions continued to be aggressive and dominant for the same length of time or longer. Bradycardia is so commonly found in B_1 deficient rats that it is now used as a method of assay for this vitamin (Williams and Spies, 1939). Judging from the possibilities of direct and severe effects on the nervous system, circulatory system, and on respiratory mechanisms (Elvehjem, 1942), it is somewhat surprising that our mice, when totally deprived of thiamin, were able to retain their dominant rank and exhibit aggressive behavior as long as they did.

Our observations on mice show some similarities with the carefully controlled experiments reported by Wilder (1943), using human subjects, who, of course, could be exposed to a much less drastic restriction of thiamin. His subjects showed marked individuality in the onset of B_1 avitaminosis, as did our mice. He reports (p. 105) "changes of behavior, marked alteration of attitude and progressively decreasing ability to perform accustomed tasks or to make social adjustments within the group." Such changes also occurred in our mice in most instances, but usually only when athiaminosis became acute. It must be emphasized that our mice were subjected to a social situation that was much simplified even for mice and was incomparably simpler than the human situation investigated by Wilder and his associates.

It is a matter of some interest to note that, with the mice, behavior changes were not nearly so sensitive an index of the onset of athiaminosis as was a decrease in weight and that changes in the social reactions involved in securing passive submission or even in mild fighting were less sensitive to loss of thiamin than were matters of body posture and of appetite for food. It has been reported by Williams and Spies (1939) that many of the pathological changes of experimental beriberi have recently been considered to be the result of starvation rather than the result of thiamin deficiency. Davison

and Stone (1937) concluded that "the pathological changes in the nervous system in animals suffering from inanition are essentially the same as those deprived of vitamin B_1, or both vitamins B_1 and B_2." These statements are of interest since our mice ate less and less as thiamin depletion progressed, and undoubtedly some of them were starving in addition to being deprived of thiamin.

Contemplation of the present experiments, especially when viewed against the background furnished by the previous work of Ginsburg and Allee (1942) and of J. P. Scott (1942, 1944), increases our respect for what may be called the "purely psychological" factors, as contrasted with other phases of physiology, involved in the dominance-subordination behavior of these mice.

The present study, like all others on related subjects from our laboratory group, was not planned or prosecuted as an indirect approach to the social life or vitamin requirements of man except as human problems form an integral part of the broad field of general animal sociology and physiology. Mice are not men; yet each displays variants of social patterns that have much in common (cf. Allee, 1945). Our group is engaged primarily in an investigation of aspects of general sociality, much as the general physiologist is concerned with the physiological properties of protoplasm. Students of general physiology and general sociology are working on the sound hypothesis that there are common attributes and tendencies that underly the physiology and sociology of all organisms and that comparisons even between widely different organisms are often profitable.

SUMMARY

1. All experiments reported here were on closely inbred black mice (C57, Jackson Memorial Laboratory). Adult males only were used. They were caged separately and were brought together in staged pair-contacts at appropriate intervals. Under these conditions the mice establish a rank-order somewhat comparable with that known in man for tennis players.

2. Controlled amounts of Merck's thiamin hydrochloride, added to an already adequate diet, produced no increase in aggressiveness or in the ability to win fights.

3. Mice that are returned to a full diet after being deprived of thiamin for 15 days or more soon show a strong upswing in social rank whether they are (*a*) meeting other mice that they have fought only a few times or (*b*) meeting individuals in a long-continued series of pair-contacts.

4. Mice that have maintained alpha rank for some time have a marked tendency to remain dominant; and, conversely, mice at the bottom of the fighting order tend to give passive submission when placed in contact with another mouse.

5. Changes occur in the rank-order for reasons beyond our control. These take place more frequently in intermediate ranks than among those in alpha or in omega position.

6. Alpha mice usually retain that status after being completely deprived of thiamin until they are in an advanced stage of B_1 avitaminosis. The mean span of retention of dominant rank by such mice approximately equaled that shown by control animals on full diet.

7. Thiamin deficiency, in most instances, did not affect aggressive behavior until the mice were so physically weakened that their ability to be actively aggressive was almost completely impaired. Twelve mice exhibited aggressive

behavior from 14 to 27 days on a thiamin-free diet, and three of these mice continued to be aggressive throughout the deficiency period and after it was terminated.

8. Social status among these mice, as measured by results from staged pair-contacts, until avitaminosis becomes too extreme, seems to be influenced more strongly by so-called "psychological" factors than by any other physiological considerations under our control.

LITERATURE CITED

ALLEE, W. C. 1945. Human conflict and co-operation: the biological background. Proc. Fifth Conf. on Science, Philosophy and Religion. New York.

DAVISON, C., and STONE, L. 1937. Lesions of the nervous system of the rat in vitamin B_1 deficiency. Arch. Pathol., 23:207–23.

ELVEHJEM, C. A. 1942. The biological action of vitamins. Pp. 1–16 in E. A. Evans, Jr. (ed.), The biological action of vitamins: a symposium. Chicago: University of Chicago Press.

FOY, J. R., and CERECEDO, L. R. 1941. Differences in the behavior of rats and mice towards deficiencies of certain members of the vitamin B complex. Jour. Nutrition, 22:439–50.

GINSBURG, BENSON, and ALLEE, W. C. 1942. Some effects of conditioning on social dominance and subordination in inbred strains of mice. Physiol. Zoöl., 15:485–506.

GOUNELLE, M. H. 1940. Action de la vitamine B_1 dans l'exercice musculaire et la prévention de la fatigue. Bull. et mém. de la Soc. méd. des Hôpitaux de Paris, 56:255–57.

HARRELL, RUTH F. 1943. Effect of added thiamine on learning. Teachers College, Columbia University, Cont. to Educ. No. 877. Pp. 55.

KEYS, ANCEL. 1943. Physical performance in relation to diet. Federation Proceedings, 2:164–87.

KEYS, ANCEL, and HENSCHEL, A. F. 1942. Vitamin supplementation of U.S. Army rations in relation to fatigue and the ability to do muscular work. Jour. Nutrition, 23:259–69.

KEYS, ANCEL; HENSCHEL, A. F.; MICKELSEN, O.; and BROZEK, J. M. 1943. The performance of normal young men on controlled thiamine intakes. Jour. Nutrition, 26:399–415.

NICE, L. B. 1912. Comparative studies on the effects of alcohol, nicotine, tobacco smoke and caffeine on white mice. Jour. Exper. Zoöl., 12:133–52.

OSBORNE, T. B., and MENDEL, L. B. 1919. The nutritive value of wheat kernel and its milling products. Jour. Biol. Chem., 37:557–601.

SANDZA, JOSEPH G., and CERECEDO, LEOPOLD R. 1941. Requirement of the mouse for pantothenic acid and for a new factor of the vitamin B complex. Jour. Nutrition, 21:609–15.

SCOTT, J. P. 1942. Genetic differences in the social behavior of inbred strains of mice. Jour. Hered., 33:11–15.

———. 1944. An experimental test of the theory that social behavior determines social organization. Science, 99:42–43.

SIMONSON, E.; ENZER, N.; BAER, A.; and BRAUN, R. 1942. The influence of vitamin B (complex) surplus on the capacity for muscular and mental work. Jour. Indust. Hyg., 24:83–90.

TAYLOR, C. M. 1942. Food values in shares and weights. New York: Macmillan Co. Pp. 92.

UHRICH, J. 1938. The social hierarchy in albino mice. Jour. Comp. Psychol., 25:373–413.

WILDER, RUSSELL M. 1943. Symptoms and signs of thiamine deficiency. Res. Pub. Assoc. Res. Nerv. and Ment. Dis., 22:101–12.

WILLIAMS, R. D.; MASON, H. L.; WILDER, R. M.; and SMITH, B. F. 1940. Observations on induced thiamine (vitamin B_1) deficiency in man. Arch. Int. Med., 66:785–99.

WILLIAMS, ROBERT R., and SPIES, TOM D. 1939. Vitamin B_1 (thiamin) and its use in medicine. New York: Macmillan Co. Pp. 411.

WINTROBE, M. M.; FOLLIS, R. H., JR.; HUMPHREYS, S.; STEIN, H.; and LAURITSEN, M. 1944. Absence of nerve degeneration in chronic thiamine deficiency in pigs. Jour. Nutrition, 28:283–88.

ZIMMERMAN, H. M. 1943. Pathology of vitamin B group deficiencies. Res. Pub. Assoc. Res. Nerv. and Ment. Dis., 22:51–79.

NEW BOOKS IN PHYSIOLOGICAL ZOÖLOGY

[This section comprises reviews of selected new books containing new and significant material in the field of physiological zoölogy. Books submitted for review should be sent directly to the review editor: Frank A. Brown, Jr., Department of Zoölogy, Northwestern University, Evanston, Illinois.]

THE PERMEABILITY OF NATURAL MEMBRANES,[1] BY HUGH DAVSON AND JAMES FREDERIC DANIELLI

Reviewed by A. K. PARPART
Princeton University

IT IS heartening, in these times, to have appear a book on the permeability of natural membranes—a book which expresses the viewpoints and individualities of its authors. Particularly striking is the fact that it comes from a country whose research workers had all but abandoned their heritage in this field.

In content it is a balanced selection of the more important facets of this problem. The reader will see the highlights of the research in this field but should critically examine the reflection from those facets which have been especially polished by the authors.

One facet that has attracted the admiration of the workers in this field is the quantitative nature of the results obtainable. The authors are not exempt from this feeling, and they have striven to see it in all its perfection. It appears true that the study of permeability has reached a semiquantitative stage, but it is well to keep in mind, while reading the book, that many of the physical phenomena which underlie the permeability properties of natural membranes have not themselves attained full quantitative stature.

Reflections from other facets of the problem have been caught and are to be seen in the chapter on "Equilibrium Conditions of Cells" and in the data chapters, viii through xvii and xx. In these chapters one finds a presentation of much of the research data that have been obtained in this field up to 1940. A few reflections were not caught, and some show imperfections.

In sharp contrast is the chapter (ii) on the "Methods of Studying Permeability," which forms the basis for the quantitative data presented in many subsequent chapters. This material deserves better attention than the authors saw fit to give it.

The other chapters contain facets which have been highly polished so that they may mirror the interpretations and theories of the authors.

The problem of membrane permeabi ity is indeed a rare gem, and it has received another setting. The authors may feel well pleased with their labors, which were numerous, if out of an admiring throng comes a further understanding of the problem.

[1] Cambridge: Cambridge University Press; New York: Macmillan Co., 1943. Pp. x+361. $4.75.